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# An In Vivo Study of the Penetrability of Endodontic Restorations

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AN IN VIVO STUDY OF THE PENETRABILITY  
OF ENDODONTIC RESTORATIONS

by

Donald L. Scorallo, D.D.S.

A Thesis Submitted to the Faculty of the Graduate School  
of Loyola University in Partial Fulfillment of  
the Requirements for the Degree of  
Master of Science

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## AUTOBIOGRAPHY

Donald Lawrence Scorallo was born in Aberdeen, Washington on the first day of July, 1935. There, he attended elementary schools and graduated from Weatherwax High School in 1953.

His college education was obtained at both Grays Harbor Community College in Aberdeen and The University of Puget Sound in Tacoma, Washington. He then entered Chicago's Loyola University School of Dentistry in 1958. His degree of Doctor of Dental Surgery was conferred in June, 1962.

Since graduating, he has served continuously on active duty in The United States Navy Dental Corps. For his first year he was designated to receive a rotating dental internship at The Great Lakes Naval Hospital. His service career has also taken him aboard The U.S.S. Sperry and The Miramar Naval Air Station, both in San Diego, California. After spending a year in Vietnam, he returned to the San Diego area for further duty.

Beginning in August, 1969, the author embarked on a three year period of graduate training. The first year was at The Naval Dental School in Bethesda, Maryland. In 1970 he was selected by the Navy and the Graduate School of his

dental alma mater, Loyola University, to pursue a dual graduate program leading to the didactic degree of Master of Science in Oral Biology and a Certificate of Specialty Training in Endodontics.

Dr. Scorallo is a career officer and holds the rank of Lieutenant Commander in The United States Navy. Shortly, he will assume the duties as the staff endodontist at Treasure Island's Naval Station in San Francisco.



## DEDICATION

To Betty, my wife and best friend, and to each of my children. Their love, loyalty and encouragement have served as an inspiration to achieve otherwise unreachable goals.

To the memory of my father, Joseph V. Scorallo, whose personal sacrifices cannot be matched but will never be forgotten.

To a compelling credo which echoes that it only takes a little more--to go first class.

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## CHAPTER I

### INTRODUCTION

It is the accepted view of the preponderance of endodontic practitioners that the ultimate goal of root canal therapy is to seal the root canal system from the periodontal ligament. Clinical practice and research has shown that all spaces of the root canal must be filled with some form of stable, inert, yet manageable material or materials. This necessity has been pointed out repeatedly since Dr. Hudson of Philadelphia reputedly filled the first "fang" with gold foil around 1830.<sup>86</sup> Since that time practitioners have sought a variety of methods to cremate, extirpate and/or obliterate the root canal and its contents.<sup>2</sup> Dentists have tried waxes, cotton, numerous varieties of cement, different metals, wood, resins, and countless technics involving gutta percha. Each contributor implied or stated unequivocally that his was the "best" or "easiest" method and surely offered the most complete manner of solving the problem of the unfilled root canal.

The early pioneers of dentistry empirically envisioned that the tissue "fluids and gases" must be excluded from the canals. This insight is expressed in the current

view that the tissues surrounding the root react pathologically when extracellular fluids do not enjoy a total biologic exchange of nutriments and wastes.

## CHAPTER II

### REVIEW OF THE LITERATURE

#### A. Methods of Testing Dental Margins

Incensed by the poor quality of the endodontic treatment being rendered by many of his colleagues, McQuillen soundly admonished them in 1867 "for the slovenly character of their operations".<sup>87</sup> Many men were still leaving the canals unfilled after removing the pulpal remains. Some years earlier he had shown in experiments on extracted teeth that water could penetrate into the pulp chamber. By sealing the foramina with wax, and placing the teeth in water for a few hours, removed them, and the previously dry pulp chamber could be found to be saturated with water. McQuillen stated, "these experiments were instituted to prove that the liquor sanguinous and other fluids in this way could enter the pulp cavities of teeth which were left unfilled as in the plan proposed above, and then decomposing become more prolific sources of trouble to the patient". These experiments clinically portrayed the thinking of many of the early practitioners and established hermetic sealing of the root canal as a pillar of endodontic success.<sup>2</sup>

Following these early efforts of testing, a variety of methods have been employed to test the sealing ability and marginal adaptation of dental filling and cementing materials. Essentially, six methods have been tried by investigators:

1. Direct Observations
2. Bacteriologic Method
3. Dye Method
4. Fluorescence Method
5. Air Pressure Method
6. Isotope Method

#### 1. Direct Observation

Individually, as well as collectively, dentists are an ingenious and inventive band. One only has to quickly leaf through any dental supply catalogue, new or old, and browse among the many instruments and devices to be assured of their ingenuity. The efforts of the early pioneers of dentistry, i.e., McQuillen, Rhein, Trego, Farrar, and Black, must be especially appreciated. They had little or no scientific precedent to guide them. There were no sophisticated testing devices nor today's modern technology at their disposal. The status and success we enjoy today has been made possible as they sought to shed light on dentistry's enigmas. Indeed, current efforts have been predicated by their ventures. In a large measure we have simply improved

or embellished upon their basic ideas.

Because of the gross instability of the restorative materials of the McQuillen and Rhein era, marginal discrepancies were commonly measured with a micrometer. As the adaptability of dental materials improved, the micrometer could not be relied upon to reveal minute cavo-surface defects. It simply provided a linear measure of the expansion or contraction of the bulk filling rather than the adaptation along its periphery.

During a presentation before the Illinois State Dental Society, in 1895, Cattell displayed the roots of six teeth he had filled by utilizing separate technics.<sup>24</sup> After sectioning the roots and examining his results, Cattell humbly admitted that it was much easier to write about filling a root canal than it was to perform the task of filling a root canal.

Later that same year Harlan invited four dental practitioners to fill root canals exercising their favorite technics and materials.<sup>57</sup> Afterward, the external root surface was filed in the vertical plane until the root canal was exposed. The quality of each man's efforts was visually judged and it was decided that "roots can be filled very satisfactorily out of the mouth by different men using their own methods".



Evaluation of root canal fillings continued to be made by direct observation. In 1931, Buchbinder filled glass tubes with a sealer which he formulated and observed them for one year.<sup>20</sup> Lantz and Perrson devised a means to take serialized x-rays over a protracted period of time to observe experimental endodontic procedures in dogs.<sup>76</sup> A Japanese dental school reported in 1970 that teeth endodontically restored in the laboratory by students were evaluated by roentgenograms.<sup>115</sup> What appeared to be poorly obturated canals roentgenographically were later substantiated by black ink penetration.

## 2. Bacteriologic Method

Quite logically, the bacteriologic method was one of the first to be applied since the teeth are perpetually emersed in a myriad of organisms. Van Leeuwenhoek, with his microscope, described the bacteria that he found in scrapings from his own teeth in 1683.<sup>22</sup> Their implication with dental caries was not established until 1880 by W. D. Miller.<sup>65</sup>

Evaluations of the efficiency of the tooth/restoration margin historically dates back to at least 1900 when dentists became concerned about their amalgam formula. It was generally felt that the life expectancy of an amalgam restoration was four years before signs of recurrent dental caries became evident. Thus, dental scientists were alerted to the necessity

of attaining impenetrable margins. Hewett proposed that capillary attraction (a result of a combination of the proposed factors in dental caries) be added to the list of the known causes of dental caries.<sup>59</sup> He showed how resin varnish could enhance the seal of amalgam and gold in a test solution of "artificial saliva".

In 1902, Webster averred that the claims for or against the use of different root canal fillings lacked scientific reason.<sup>136,137</sup> He utilized salivary bacteria in a series of experiments to test the sealing qualities of several temporary access cavity sealers and root canal filling materials placed in the ends of drawn glass tubes. He found that all the sealants tested could be penetrated by normal oral flora. It was concluded that few, if any, root canal fillings are resistant to either moisture or bacteria.

Due to the lack of anatomical uniformity found in the pulpal spaces, a desirable property of root canal filling material is that they be plastic or moldable upon insertion. It is also desirable that once the material is manipulated by physical force, heat or solvents, it will remain stable in its final molded form, free of elastic strains. Little, if any controlled research has been done to establish the physical characteristics of root filling materials since Price presented his findings at the National Dental Association meeting in

Chicago in 1918.<sup>106</sup> He asked several dentists to fill the root canals of pre-sterilized extracted human teeth with different materials, employing different methods and then placed them in a streptococcus media. He observed that none of the materials or methods used could adequately exclude the organisms from penetrating between the restoration and tooth.

Since then, specific microorganisms have been used to evaluate the marginal integrity of temporary fillings useful to dentists. Fraser used *Escherichia coli*, alpha hemolytic streptococci, and *Bacillus proteus* while Parris and his co-workers used *Sarcina lutea* and *Serratia marcescens*.<sup>41,100,101</sup> Grossman employed roughened glass capillary tubes to simulate root canals in order to study the comparative seals of temporary stopping, base plate gutta percha, zinc oxyphosphate cement, "Pro-Tem", and zinc oxide-eugenol cement in the presence of common oral bacteria, saliva and dyes.<sup>53</sup> The glass allowed him to view the results directly. His observations led him to suggest the placement of a "double seal" between endodontic appointments with zinc oxide-eugenol cement as the surface sealant.

The marginal adaptation of amalgam, silicate and resinous coronal restorations have also been assayed according to their relative penetrability by a variety of organisms including *Pseudomonas aeruginosa*, *Serratia marcescens*,

Bacillus globegii and Staphylococcus aureus.<sup>110,120,74,93</sup>

While bacteriologic methods of appraising the tooth-restoration interface are valid, Kapsimalis, et al, have cautioned that the sterilization technics employed with extracted teeth can lead to false positive or false negative findings.<sup>72</sup> They suggest three factors that could result in such erroneous conclusions: 1) The act of sterilizing the tooth surface, although necessary, may alter the internal tooth environment rendering it incapable of sustaining bacterial growth; 2) Bacteria already present on the surface may contaminate the results unless completely eradicated; and 3) The filling substance under investigation may exhibit antibacterial activity and destroy the test organisms.

### 3. Dye Methods

Dyes have been widely employed as a means of indexing and comparing the sealing ability of dental restorative materials. Many investigators hypothesize that if a material will seal against a dye not only can the character of penetration be easily observed, but it may also be expected to marginally oppose the ingress of saliva and bacteria.

Prior to using saliva and bacteria, Webster employed a dye in his penetration experiments.<sup>135</sup> He fabricated fine glass tubing and filled them with bulk root canal fillings widely used at that time, i.e., chloropercha plus gutta percha

cones, gutta percha alone, chloropercha plus cotton, and three different cements. The ends of the tubes were placed in a "red colored solution" for 24 to 72 hours. Although the chloropercha plus gutta percha gave the best results, all the materials as tested leaked at their periphery. He also observed that his root canal fillings made by heating and packing gutta percha cones exhibited considerable leakage due to contraction upon cooling.

In 1939 Grossman also used glass tubes to test a number of temporary fillings against the penetration of several dyes.<sup>53</sup> Of the materials he included in this study, zinc oxide-eugenol was the only one which did not leak. He tried methylene blue, gentian violet, carbol fuchsin and Sudan III and noted that the choice of dye had no effect on his results.

In 1950 Tremblay felt that the cold-curing acrylics offered possibilities as a restorative material.<sup>130</sup> This brought about much controversy since the instability of resins to thermal changes was widely accepted. Fisher, Castagnola, Buchanan, Kornfeld, Hirsch and Weinreb used dyes to evaluate the early resin formula known during the 1950's.<sup>40,23,19,73,62</sup> Although not as effective a sealant as amalgam, they generally found the self-curing resin superior to silicate against prontosil soluble red, methylene blue, barium sulfide and

aniline blue dyes.

Massler and Ostrovsky also packed temporary and permanent type restorative materials into glass tubes having an inner diameter of 5 mm.<sup>83</sup> Above each material, cotton was seated. The tube's ends were submerged into solutions of gentian violet or methylene blue. The length of time it took to stain the cotton was then recorded for each tube. The gutta percha, temporary stopping, zinc oxyphosphate cement and self-curing plastic leaked within a matter of hours while amalgam sealed the best, followed by zinc oxide-eugenol cement, then gold inlays.

Later in the same decade, Schroeder and Stewart assessed commonly used root canal sealers against a solution of methylene blue.<sup>117,118,123</sup> They reported that AH 26, Diaket, Kerr's and Grossman's sealers all gave good results.

In 1960 Going preferred a combination of dye (crystal violet) and radioactive sodium iodide ( $I^{131}$ ) when comparing commonly used restorations in operative dentistry.<sup>46</sup> He also sought to determine if molecular size made a difference in the diffusion of solutions. Although both solutions provided satisfactory conditions for study, the isotopes demonstrated greater penetrability than the dye. The development of autoradiographic technics allowed the detection of minute amounts of tracer ions which would not otherwise be

evident.

Parris and Kapsimalis attempted to evaluate the relative efficiency of temporary stopping gutta percha, base plate gutta percha, zinc phosphate cement, zinc oxide-eugenol cement, cavit and amalgam as temporary sealing agents in extracted anterior teeth.<sup>99</sup> The teeth were subjected to 10 cycles of 60° C. water for one minute followed by a 4° C. media of 2% aqueous aniline blue dye. Following these abrupt changes in temperature, only cavit and amalgam exhibited leak-proof cavity seals. Also trying aniline blue, Kakar and Subramanian filled roughened glass tubes 4.5 mm in diameter and extracted teeth with cohesive gold foil, amalgam, gutta percha, zinc oxide-eugenol, zinc oxyphosphate and silicate cements.<sup>69</sup> Nearly all of their restorations and seals showed leakage within 48 hours, however, amalgam and zinc oxide-eugenol appeared to be best. In addition, their feeling was that glass tubes provided unreliable results under these conditions.

Taking another tack, Pinto and Buonocore endeavored to magnify their results by testing the effects of having a base or cavity liner under the commonly used operative dentistry materials placed in bovine teeth. The penetration of Basic Fuchsin or 0.5% chromotrope dye was measured.<sup>105</sup> They found that marginal permeability increased or remained

the same when liners or bases were used except when placed under silicate cement restorations.

The type of solution that the teeth are stored in prior to a penetration study may affect one's results. Lyell, Barber and Massler deposited extracted teeth in saliva, sodium sulfide, tap water, distilled water and moist air for varying time periods and concentrations.<sup>80</sup> Initially, unlined and unbased Class V amalgam restorations had been placed in the extracted teeth. This was followed by storage in the different solutions. Next, the restorations were exposed to combinations of 3.8% toluidine blue plus S<sup>35</sup> as sodium sulfate and the toluidine blue combined with Ca<sup>45</sup> as calcium chloride. They reported that the in-vitro submersion of restored teeth in sodium sulfide or saliva will chemically block the ingress of dyes and isotopes. They also agreed with Going's observation that isotopes penetrate further than dyes. Unfortunately their work did not include the affect that solutions of formalin or saline may have had under the same experimental conditions.

While seeking to evaluate the clinical possibilities of zine oxide-creosote ("Nobudyne"), Sekine and coworkers also used a glass tube method of testing marginal integrity.<sup>119</sup> They compared its seal to gutta percha and copper cement against methylene blue. The "Nobudyne" fared the best of these three



materials as a sealant and was recommended for use as an "ideal direct pulp capping material" or as a root canal filling.

The tenor of nearly all penetration studies performed in research laboratories would lead us to believe that all dental restorative materials leak to one degree or another. Yet daily, as we clinically remove coronal restorations which may be even 5 to 10 years old, we see that the underlying cement base appears to be completely dry. These were the thoughts of Roydhouse, Weiss and Leonard as they proposed to explain why laboratory research failed to reflect the clinical situation.<sup>112,113</sup> Three sets of experiments were performed on extracted bovine and human teeth restored with acrylics, silicates and amalgam. These were evaluated by several dyes (basic fuchsin, methyl violet and chromotrope 2R). The interpretation of their results led them to a number of profound observations. Among them were: 1) Success is affected by a. the ability of an operator, b. the restorative material, c. the test solution's ionic charge, molecular size and solvent, and d. presence of viable pulp (which influences osmotic, gaseous and vapor pressures); 2) The significance of laboratory research must be looked upon as a "potential" rather than a clinical reality.

Antoniazzi, Mjor, and Nygaard-Ostby focused their attention on the in vitro sealability of Kloroperka N-O, AH 26, and Kerr's sealer with a 0.5% aqueous solution of methylene blue adjusted to pH 7.<sup>4</sup> Similar to Roydhouse, they concluded that in vitro studies offered little information which could be applied biologically.

In 1968, Curson and Kirk assessed various aspects of root canal cements including their seals.<sup>35</sup> Pairs of 1 mm diameter glass capillary tubes filled with each sealer were suspended for different lengths of time in methylene blue. They found that ZOE, Rickert's, Grossman's, AH 26, Diaket and Tubliseal sealers were all satisfactory. It was also observed that the seal obtained by Grossman's formula degenerated after 30 days. Quite obviously, determinations based on prolonged test periods is in order if dye studies are to have validity.

#### 4. Fluorescence Method

The applications of the principles of fluorescence in industry and in biologic research have been known for many years. In 1929, the dye fluorescein was given orally to mark the extent of cancerous tissues which were to receive therapeutic radiation.<sup>33</sup> Since it is non-toxic, it has been widely administered intra-venously to detect and combat neoplastic growths throughout the body.<sup>75,96</sup>

It is quite probable that Nelson, Wolcott and

Paffenbarger were the first to use flourescein in dentistry.<sup>95</sup> Wolcott's thesis was to determine the clinical aspects of marginal percolation around dental restorations. Using a pyrometer on live subjects they found that coffee ingested at 60° C. would raise the temperature under cold-cure resin fillings to 52° C. Likewise, a cold beverage (4° C.) could lower the intaglio surface temperature to 9° C. Hence, a 43° C. range of temperature is possible during a meal beneath a resin restoration. Since the linear coefficient of expansion of dental restorations is generally greater than that of tooth structure, marginal discrepancies must occur daily wherever such restorations exist. For example, the coefficient of thermal expansion for quick-cure acrylic has been approximated at seven times that of tooth structure. Thus, resin fillings could be expected to leak marginally on an expansion-contraction basis alone. To test this hypothesis Wolcott put crystals of fluorescein dye on the pulpal floor of a prepared cavity. After filling the cavity with a direct resin material and allowing it to set, the tooth was subjected to 10 cycles of chilling and warming. With the aid of ultra-violet light and a microscope, he noticed fluid exuding from margins showing the yellow-green fluorescence characteristic of the dye. Although pertinent data was not included in the article, the authors concluded that temperature changes cause fluids to

escape around the margins of restorations of gutta percha, zinc oxide-eugenol cement, silicate cement, zinc phosphate cement, amalgam, cast gold, gold foil and acrylic resin.

Christen and Mitchell have delved extensively into the background and application of ultraviolet radiation and fluorescence.<sup>26,27</sup> Three fluorescent dyes (rhodamine B, fluorescein, and demethylchlortetracycline) were used to determine microleakage potentials around amalgam, with and without cavity varnish liners, as well as zinc oxide-eugenol fillings and gutta percha restorations in bovine teeth. An amalgam lined with cavity varnish was the most resistant to penetration while the zinc oxide-eugenol restoration could not be evaluated by fluorescent dyes. In a preliminary trial, fluorescein was mixed separately with eugenol, then with zinc oxide powder. The inherent fluorescence of the dye was not visible. Therefore, it would appear that the use of fluorescent materials to detect marginal discrepancies for any material containing zinc oxide powder or eugenol may be of limited value if Christen's observations are correct.

Holliger has devised an elaborate photography arrangement to observe fluorescent microleakage around silicates and amalgams.<sup>63</sup> He has been able to obtain macro-photographs ( 5 x magnification) of his tooth sections for detailed scanning. He used an interesting combination of

orange and red rhodamine called "Red Water Glow".

Gross, Goldberg, Loiselle and Stuever used a 2% solution of fluorescein dye to penetrate amalgam restorations in a series of experiments involving animal and human subjects.<sup>52,79</sup> Their purpose was to compare in vitro and in vivo data to determine whether in vitro findings may be looked upon as having clinically valid significance. Unlined amalgams were placed on the buccal aspect of molars in live young Syrian hamsters. Four weeks later, the animals were re-anesthetized in order to apply the dye locally over the teeth for five minutes duration. In addition, amalgam restorations were placed in extracted teeth from hamsters and similarly bathed in fluorescein. This allowed the authors to evaluate the restorations placed: 1) in vivo and tested in vivo, 2) in vivo and tested in vitro and 3) in vitro and tested in vitro. In their human study, unlined amalgam restorations were manipulated into bilaterally paired teeth in 10 patients. On one side of the mouth the teeth were bathed in the dye after allowing the amalgam to age for two weeks. From the remaining side the teeth were first extracted, then placed in the dye. From this they determined that the mean leakage score for fillings placed and tested in both animals and humans was 0.2. In vitro, the mean human penetration score was 2.5 compared to the hamster's 2.3.

Thus, the marginal penetration of amalgam restorations is about twelve times greater in extracted teeth than in viable ones when tested with fluorescein.

In Messing's view, commercially prepared fluorescein leads to false impressions because of its high penetrability due to its low molecular weight.<sup>91</sup> He produced a high molecular weight dye by dissolving fluorescein isothiocyanate in rabbit gamma globulin. He then filled pairs of canals with silver tips and/or gutta percha in combination with chlorapercha, AH 26 or Rickert sealers. The comparative seals affected by these root canal filling technics were judged by placing his conjugated dye either within the root canal or dipping the apices of the teeth into it. Under these conditions, all of his root canal fillings effectively resisted the dye. This prompted Messing to hypothesize that regardless of the filling method preferred, a good hermetic seal will result when a careful filling technique is adopted following adequate biomechanical canal preparation. Here, one must also speculate on the reliability of fluorescein in view of Christen's observation and the zinc oxide-eugenol contents of many root canal sealers.

Another means of evaluating canal obliteration was tried by Ainley on extracted human teeth.<sup>1</sup> He questioned whether chilling gutta percha and silver cones would produce

superior marginal fittings. He filled the apical 5 mm of each canal with the test filling material with and without Diaket root canal sealer. It is of interest to note that in a preliminary study, Ainley decided to use Diaket rather than Roth 801 (a Grossman formula) as his sealant because the latter fluoresced when mixed with his test dye rhodamine B. Apparently the Roth 801 formula did not mask the fluorescence like unadulterated zinc oxide-eugenol did for Christen.<sup>26</sup>

Ainley carefully sealed the rhodamine B in juxtaposition to the various fills. He endeavored to detect any fluorescence in the distilled water surrounding his specimens with a fluorometer. He stated that: "No determination could be made of a superior obturating technique because of the overlapping range of leakage values between the test groups and the broad inconsistent range of values within a given test group."

Most recently, Geiser and Jensen have compared the relative seals of Kerr's sealer, Kerr tubli-seal, Diaket, and AH 26 by exposing them to a 2% fluorescein solution and a bacteriophage for seven days.<sup>42</sup> The dye was placed either within the canal or at the tooth's apex. It was concluded that none of these canal cements provided a perfect seal against fluorescein. However, Diaket and AH 26 were at least 97% effective with the bacteriophages. When these

results are viewed in the light of present day clinical success, the authors claim that a fluid tight seal is apparently not necessary in endodontic practice.

#### 5. Air Pressure Method

William E. Harper was typical of the early imaginative breed of dental investigator. Harper devised a method of testing cavity retention features and insertion techniques for amalgams by the use of air pressure.<sup>58</sup> He filled steel test tubes with amalgam. An air-tight connection was made with the underside of this tube connecting it to an air pressure guage and air tank. The amalgam/tube was immersed in a water bowl while the air valve was opened. The amount of air pressure needed to evoke air bubbles was recorded for 400 samples. Like Roydhouse, Harper concluded that if he had to restrict his opinion on the basis of his experiments alone, amalgam would be condemned! However, the history of amalgam's clinical dependability directly refuted this temptation. The amount of air pressure a filling must withstand to remain "air-tight" under the conditions of the mouth would have to be established.

Fiasconaro and Sherman adopted Harper's method to test the sealing properties of acrylic compared to gold foils and inlays, amalgam, silicate and zinc oxy-phosphate cement.<sup>39</sup> Class V preparations of standardized dimensions were cut in



the crowns of extracted human molars and then filled with the selected materials. The root portion was resected so a brass tube could be sealed into the pulp chamber. A control valve allowed an increase of one pound of pressure every 10 seconds at the pulpal surface of the restorations. The acrylic fillings collectively leaked when 2.8 to 10.0 p.s.i. of air pressure were applied. The authors admitted that a material may be effective even though not able to survive pressures of less than one pound since no standard is available to indicate minimum pressures which could be expected to safely seal a cavity. In an addendum study, gutta percha was also similarly tested as a coronal restoration. It showed no sign of leakage up to 50 p.s.i. of air pressure.

In England fifty years later, Pickard and Gayford scorned the use of dyes and isotopes to evaluate operative restorations because they felt it would be difficult to interpret their mode of penetration.<sup>104</sup> They devised a clever method of studying leakage in extracted teeth with air pressure. The authors developed a formula which enabled them to calculate the radius of leakage paths that appear over a period of time. From this they could compute the total leakage at the periphery of a restoration.

## 6. Isotope Method

A technique of tracing a chemical element in living tissues was revealed by Chievitz and Hevesy in 1935.<sup>25</sup> They injected a solution containing radioactive phosphorus uptake and distribution in bones and teeth. Their efforts helped to broaden the avenues of biologic experimentation. It can be assumed that staking isotopes is one of the methods most widely used today for probing the biochemistry of living tissue.

Wasserman, Blayney, Groetzinger and DeWitt used the same idea as above, but confined their search to the different possible pathways of phosphorous into and within the dentition.<sup>133</sup> Their work revolved around three postulated metabolic pathways for phosphorus in teeth: 1) The pulp (its role in nutrition was firmly established); 2) The cemento-dentinal junction (the cementum was considered as part of the periodontal tissues, leaving the cemento-dentinal junction as a tooth boundary); and 3) the outer enamel surface (substances which may be absorbed from the saliva).

Maxillary anterior teeth in dogs served as their model. The pulp (as a pathway) was isolated by either injuring it or completely removing it and packing the canal with gutta percha. To eliminate the enamel surface from the system, specific teeth were covered with temporary crowns. The

cemento-dentinal junction was considered highlighted when a tooth's canal was hermetically sealed and its crown covered by a cemented temporary form. A radioactive solution of sodium phosphate containing  $P^{32}$  was injected intravenously following the dental procedures. The animals were sacrificed after 24 hours and the teeth surgically removed.

The amount of radioactivity of each crown or root was measured by a Geiger-Mueller counter and contrasted with untouched adjacent teeth. Their data indicated that the pulp was the primary locus of tooth metabolism. Also, when the pulp is non-viable, minerals can not only permeate the cemento-dentinal boundary, but can be found in the enamel as well. So, the uptake of phosphorus from saliva by the enamel is negligible compared to that taken from dentin. This finding was verified in 1943 by McCauley and Gilda, who prepared autoradiograms after injecting dogs with a  $P^{32}$  solution.<sup>84</sup>

The original autoradiograms were probably made at about the turn of this century.<sup>139</sup> Although they are widely used to solve the mysteries of histochemistry, they remain an imperfect tool inspite of recent advances in photography and biological imprints.

When "reading" radioactivity in teeth, several factors should be considered. The radioactive form of numerous elements

exists naturally in minute amounts in the teeth. Naturally occurring potassium ( $K^{40}$ ), carbon ( $C^{14}$ ), and tritium ( $H^3$ ) have been found in autoradiograms of human teeth.<sup>64</sup> Their presence may taint the findings of an experiment since radioisotopes of different elements are not readily distinguished on a autoradiogram.

In penetration studies both chemical exchanges and/or biologic absorbtions take place. Also, in all living tissues we can expect an exchange of elements to occur. For example, calcium, phosphorous, hydrogen and sulfur are incorporated in a tooth's matrix, existing in a dynamic state of equilibrium. An injection of a solution containing any one such isotope would be absorbed throughout the calcified structure. It is evident that this phenomena would encumber attempted in vivo penetration studies of radicular margins.

Selecting an appropriate isotope requires some consideration.<sup>15</sup> Not only must molecular size and half-life be considered, but also the type and range of the emitted particles is important. Radiation in the form of short acting beta particles will give sharper autoradiograms in contrast to long range beta particles and gamma rays which produce "halos and shadows". Having limited exposure range, it is necessary that the entire specimen be intimate with the emulsion for accurate measurements.

Other related factors in producing legible radioautograms have been investigated by Kapsimalis, Evans, and Tuckerman.<sup>71</sup> These men compared various concentrations of different isotopes, length of exposure time, grain size in an emulsion, and specimen thickness to determine how each affected the sharpness of a picture. Anyone contemplating radioautogram studies would benefit from reading their articles.

The effects of tissue fixation and preparation must be kept in mind. They may interact with the "fixed" isotope and leach it from the specimen. Furthermore, the interpretation of autoradiograms when testing marginal sealing is limited to the plane in which the tooth is cut. The autoradiograph must therefore be considered as valid only for the plane in which the restoration is sectioned.

Among the more mundane facets of isotope research, we should mention that the cost is high for concentrated solutions of suitable isotopes for penetration studies. To inject a laboratory animal of modest size with sufficient concentrations may well be prohibitively expensive. Another consideration is that the Atomic Energy Commission requires that all animal waste products be monitored and properly disposed of. If an animal is to be maintained for prolonged observation periods, suitable waste control measures must be

available. These are but a few of the complications which must be dealt with when using radionuclide substances.

Various isotopes have been utilized to analyze dental materials which have added to our store of knowledge. Radioactive calcium ( $\text{Ca}^{45}$ ) has been the isotope of choice for several authors seeking to evaluate the marginal stability of routine restorative materials.<sup>5,18,34,88,103,116,125,126</sup> The basic approach of these scientists was to restore extracted teeth, coat the root surface to avoid absorption through the cementum, then, bathe the restoration in calcium solution for 48 hours or less. In summary, their conclusions were: 1) In time,  $\text{Ca}^{45}$  will penetrate between the tooth and any filling material; 2) Amalgam restorations become less penetrable as they age; 3) The leakage pattern varied from silicate restoration to silicate restoration; 4) 24 hours should pass before finishing silicate restorations; 5) The best margin for resin restorations is obtained by the combined bulk-pack/flow technic; 6) It is not necessary to wait 24 hours to finish acrylics; 7) Use of cavity varnish beneath restorations enhances their marginal seal; 8) Thick mixes of zinc phosphate cement are less penetrable than mixes of thin consistency; 9) Zinc oxide-eugenol cement is a very effective seal initially but deteriorates after one month; and 10) Less penetration occurs when the prepared cavity walls have

roughened surfaces.

$\text{Ca}^{45}$  has also been used to test root canal sealers. Higgenbotham studied the sealing ability of Rickert's formula, Tubli-seal, Diaket, Procosol, and Klorperka N-O when used with gutta percha.<sup>60</sup> He tried each combination as a coronal restoration and as a root canal filling. The Kerr and Procosol formulas provided the best barriers to  $\text{Ca}^{45}$  and it improved with time. The Klorperka sealant allowed the greatest ingress of ionic fluid.

Wainwright and his associates have accomplished numerous dental projects with isotopes. Similar to Messing, he produced his own test solution by incorporating radioactive iodine ( $\text{I}^{131}$ ) with human serum albumin in an effort to increase its molecular structure.<sup>132</sup> He compared its penetrating ability to a lower molecular weight solution of sodium iodide, also containing  $\text{I}^{131}$ , on amalgam restorations in extracted teeth. Although microleakage was evident with both iodized liquids, that of the serum solution was definitely less pronounced.

A classic article in the endodontic literature was written by Dow and Ingle in 1955.<sup>38</sup> They wanted to determine if  $\text{I}^{131}$  could seep into root canals whether they were carefully or poorly obturated by the lateral condensation technic. Their autoradiograms showed that a root canal should be

completely sealed if it is to preclude apical fluids.

Talim and Singh also utilized radioactive iodine to evaluate endodontic fillings manipulated both in vitro and in vivo.<sup>128,129</sup> Extracted anterior teeth were filled with gutta percha alone or combined with zinc oxide-eugenol cement or chloropercha; silver cones with zinc oxide-eugenol, and N<sub>2</sub> alone. Six of each type were immersed in the isotope solution immediately after restoration or 1 week later. In an additional experiment teeth were similarly treated in situ and left for 1 week before extraction. Agreeable results were obtained autoradiographically from both studies. Increased penetration took place on specimens permitted to sit for one week regardless if they were filled in vitro or in vivo. Gutta percha or silver points with zinc oxide-eugenol and N<sub>2</sub> alone effected good seals. Gutta percha by itself or combined with chloropercha were the least effective of those tested.

Brannstrom, Bergman and Soremark utilized Na<sup>22</sup> in saline to explore the margins of lined and unlined amalgam restoration.<sup>10,16</sup> Their observations differed from Phillip's and Swartz's findings in that their copalite-lined restorations did not oppose the Na<sup>22</sup> adequately. A dentin coating of a Chamber-type varnish consisting of 5 parts calcium hydroxide, 5 parts zinc oxide, 2 parts polystyrene and q.s. chloroform



resisted or reduced the penetrating sodium ions even when the amalgam restorations were subjected to temperature changes. This also suggests the possibility that  $\text{Ca}^{45}$  and  $\text{Na}^{22}$  do not have equal penetration capabilities as Going and Massler pointed out in a later article.

Going, Massler, and Dute tried a number of different radioactive isotopes ( $\text{S}^{35}$ ,  $\text{P}^{32}$ ,  $\text{Na}^{22}$ ,  $\text{Rb}^{86}$ , and  $\text{Ca}^{45}$ ) to decide whether ionic charge and chemical activity affected their ability to invade the restoration/tooth interface.<sup>44</sup> Class V preparations were cut in 147 extracted teeth, restored with different materials, then immersed for 24 hours in the radioactive solutions. Their findings were declared after observing the individual autoradiograms and comparing them to the penetrations seen in unrestored preparations. The authors discovered that, of the isotopes selected,  $\text{P}^{32}$  was the only one which did not reach the pulp chamber when placed directly into the open cavities. The  $\text{S}^{35}$  and  $\text{Ca}^{45}$  resulted in the greatest degree of penetration and also produced the most sharply detailed autoradiograms. In general, the negatively charged isotopes,  $\text{S}^{35}$  and  $\text{P}^{32}$ , were absorbed in greater abundance on the restoration surface. Depending on the isotope selected, the order of leakage among silicate, zinc phosphate and zinc oxide-eugenol cements could be reversed. It was concluded that the ionic charge and chemical reactivity

of the isotope, as well as the physical and chemical activity of the restorative material influence the character of the marginal penetration. In a later paper with Massler, Going postulated: "Penetration appeared to be a function of chemical reactivity rather than of particle size or ionic charge, since positively charged sodium ( $\text{Na}^{22}$ ) with an atomic weight of 22 was the deepest penetrator, whereas calcium ( $\text{Ca}^{45}$ ) with an atomic weight of 45 and also positively charged, was the poorest penetrator."<sup>45</sup> Yet, sulfur and iodine, with atomic weights of 99 and 131, were the most reliable penetrants.

Several different isotopes were further studied by Marshall and Massler to evaluate various endodontic filling materials and technics.<sup>82</sup> Gutta percha and silver points were packed solely or in combination with Kerr's, Wach's Kloroperka N-O, or Grossman's cements into the apical third of extracted human teeth. The canals of these teeth had been instrumented to where a size 6 Kerr file could pass through the apical foramen. The integrity of these endodontic fills were checked by placing the tagged solutions either within the canals or outside their apex. As in Going's work, Marshall found sulfur<sup>35</sup> provided the best autograms. With only minor differences, all his sealers and root canal fillings defied entry to the activated ions.

Kapsimalis and Evans carefully selected radiosulfur ( $S^{35}$ ) because it was an anion, tritiated glucose since it is nonpolar and tritiated proline because of its chemical polarity and ability to act as an acid or base depending on the pH of its solution.<sup>70</sup> These were utilized to compare the sealing properties of commonly used endodontic materials. Extracted teeth were prepared to a #90 file, then filled with gutta percha or silver points alone or with one of several root canal sealers. In their study the tooth surfaces were not coated and the teeth directly submerged into the tracer solutions for 48 hours. Horizontal sections, 300 microns thick, were cut and autoradiographs made of these serial segments. Gross leakage appeared when the canals were filled with gutta percha cones or silver points alone. Kloroperka N-O, Kerr's sealer, Diaket and PCA sealer leaked with all of the isotopes used in this study. Only Procosol and AH 26 root canal cements showed no leakage against these three radioactive forms.

More recently, Going, Myers, and Prussin used radioactive manganese ( $Mn^{56}$ ) to formulate a means of quantitating microleakage of non-metallic dental restorations.<sup>47</sup> Manganese was selected because its presence would not be masked by other isotopes potentially present when measured by a scintillation detector. They compared temporary stopping,

resins, and silicate cements placed in human teeth both in vivo and in vitro. In general, the authors found the in vivo uptake to be greater than in vitro uptake at the margins. Of interest here was the authors' observation that in vitro, temporary stopping provided a good initial seal. The same material in vivo absorbed approximately six times more tracer solution (higher values indicated a poorer seal). Since the coefficient of thermal expansion of gutta percha is greater than that of the tooth, a fact which may be related to the lack of marginal seal in vivo.

Radioactive phosphorus ( $P^{32}$ ) was used most recently in Italy by Barlotta, Negro, and Roccia.<sup>7</sup> They compared zinc oxide-eugenol, Wach's, Kerr's, Grossman's and chloropercha pastes using a variety of filling methods. They confirmed the finding of others before them, that none of those tested totally precluded the entry of the test solution.

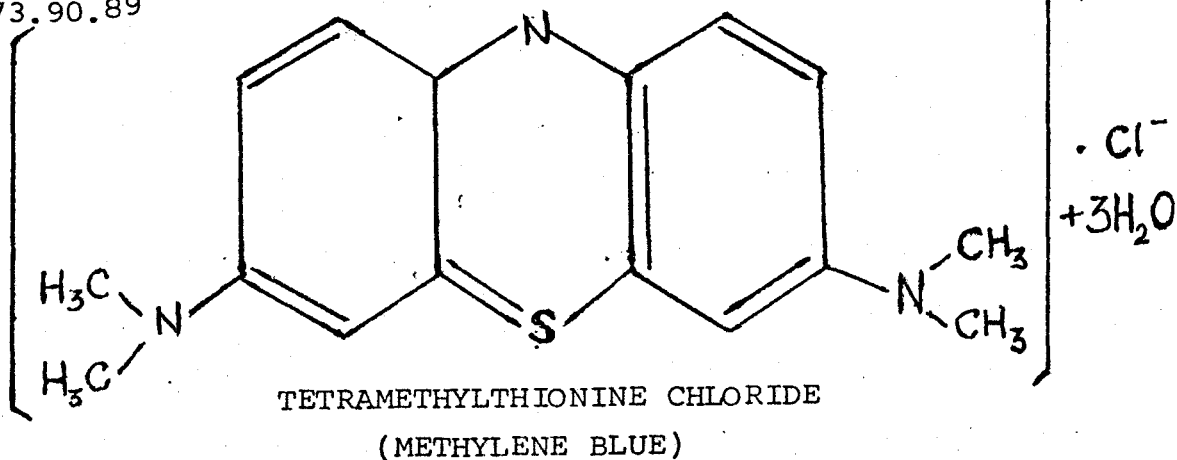
Combinations of isotopes with dyes have been concocted to study the adaptation of dental restorations in order to determine how molecular size influences the degree of penetration. Going and his associates tried  $I^{131}$  combined with crystal violet dye to check all the coronal restorative materials commonly in use today.<sup>46</sup> Lyell's group tried mixing toluidine blue with either  $Ca^{45}$  or  $S^{35}$  to analyze amalgam restorations.<sup>80</sup> Both studies agreed that the

isotopes penetrated much deeper than the dyes.

### B. Methylene Blue

A dye was selected for this investigation because its molecular size and structure is more realistically applicable than smaller structured ionic tracers. It was postulated that if chemical attraction occurred between the dye solution and the tested area and/or material, that combination of molecules would possibly be visible. More importantly, if it did take place, it would represent potential penetration by a molecular structure ten times the size of what presently appears to be the most suitable isotopic marker, radioactive sulfur ( $S^{35}$ ).

Methylene blue chloride was chosen in view of its relative molecular size. Its empirical formula,  $C_{16}H_{18}N_3SCL \cdot 3H_2O$ , represents a molecular weight of 373.90.89



As an organic salt it enjoys a high degree of staining ability, yet is relatively non-toxic. The positive and detracting attributes have been broadly documented in the literature. Similar documentation for other dyes is comparatively scanty.

Bacteria were ruled out as a yardstick because of their relative size. The average diameter of commonly found oral microorganisms is 2-4 microns which is approximately 10,000 times that of  $S^{35}_{56}$ . Air tests appeared to be not only technically impossible to set up in vivo, but open to personal interpretation since there is no comparable situation in the mouth with which to draw conclusions.

From the preceding, it is readily apparent that merely the selection of a test method presents a dilemma. Selection of a single test solution compounds this dilemma tenfold. The ultimate choice is the filtrate of a series of compromises. The drawbacks of the current margin-testing methods have briefly been mentioned. An in vivo experiment to evaluate endodontic materials presents special problems. When introduced into a laboratory animal, the substance should go to the periodontium and be able to diffuse into the extracellular fluid in sufficient quantities to be detectable in

some manner. It must also have the potential to permeate the apical foramina, yet not become complexed as part of the tooth structure in the process. Ideally, such a test agent must be capable of crossing the capillary barrier into the connective tissue spaces and extravascular fluids.

Bergman compared the diffuseability of positively charged (cationic) dyes (toluidine blue, methylene blue, crystal violet and methyl green) with anionic dyes (congo red, Evans blue, and methyl orange).<sup>9</sup> Their diffusion through cartilage and a dialyzing membrane was examined. As a group, the cationic dyes and methylene blue proved to be the most reliable penetrants.

Compared with other frequently used disclosing solutions, methylene blue has fared extremely well when scrutinized in vivo or in vitro within dentin or enamel.<sup>3,14</sup> The works of Bodecker and Lefkowitz in this regard are widely known and held. They were among the first to point out the different character of diffusion between in vivo and in vitro situations.

Methylene blue may be referred to as Tetramethylthionine chloride, methylene blue chloride, or Swiss blue. It is available in two other forms commercially. Methylene blue thiocyanate is used by the dairy industry to check the rate of reduction by bacteria in milk. A zinc form of

methylene blue is known but finds little applicability due to its highly toxic nature. Conn states that the pathologist and bacteriologist would have a difficult time trying to get along without this dye.<sup>30</sup> Next to hematoxylin, it is employed for a greater variety of purposes than any other biologic stain. In the laboratory it may find use as: 1) a nuclear stain histologically; 2) a bacterial stain in identifying diphtheria organisms; 3) a vital stain for nervous tissue; 4) a blood stain; and 5) an oxidation-reduction indicator in milk. Medically, it is used to treat methemoglobinemia in humans and animals.<sup>13,89</sup> It may also find use as an antidote for cyanide poisoning.<sup>43,11</sup> Generally, it is kept on hand during open heart surgery to check the patency and integrity of vessels or ducts.<sup>51</sup>

Methylene blue (Tetramethylthionine chloride) was reputedly compounded in 1876 by Caro.<sup>89</sup> As early as 1890 it was utilized therapeutically as an antiseptic in the intestinal and genitourinary tracts. Since then, it has also been recorded as an oral rinse in Vincent's infections because of this mild germicidal activity.<sup>98</sup>

In veterinary circles methylene blue is the drug of choice in treating methemoglobinemia.<sup>11,29,90</sup> Farm animals are subject to this malady due to the intake of nitrate or nitrite salts in their forage or well water. The farmer and



his family are also susceptible because of the common water supply. Internally, these salts oxidize the (normally) divalent iron in hemoglobin to methemoglobin, the trivalent state. This decreases the oxygen-carrying capacity of red blood cells. If acutely affected, an animal may die due to the resulting hypoxia. Because of its oxidation-reduction capacity, methylene blue is given parenterally or intravenously to reduce the methemoglobin back to hemoglobin.

Superficially the use of methylene blue to treat methemoglobinemia seems to present a paradox since early investigators also injected this dye to experimentally induce this condition. Depending on the dosage, some workers did and some did not achieve this end.<sup>17,94,124</sup> We now know that when perfused in high concentrations, the ferrous ion of hemoglobin is converted to the ferric form in methemoglobinemia. Low concentrations (1-4 mg/kg) induces the reversion back to the physiologic ferrous state. Bodansky has authored an excellent article covering the aspects of methemoglobinemia including a comprehensive review of the literature.<sup>12</sup>

In laboratory animals this dye may be employed to demonstrate oxidation-reduction phenomena. Anemia may also be studied since prolonged administration of this dye produces an erythrocytopenia.<sup>51,107,92</sup>

Experimentally, methylene blue has been given to a variety of animals in a wide range of doses. Rentsch and Wittekind administered it to guinea pigs, dogs, cats, rats, mice, and rabbits in concentrations ranging from 20 to 200 mg/kg in each category of animal.

It has similarly been used in man and its physiologic effects recorded.<sup>17,131,50</sup> Nadler injected 50 cc. of a 1% solution intravenously into 18 human volunteers to evaluate its clinical possibilities with cyanide poisoning. Only minor side effects were reported. However, Goluboff and Wheaton diagnosed toxic effects in the form of hemolytic anemia when using methylene blue to treat well water-induced cyanosis in 2 to 4 week old infants.

Macht and Harden were interested in its toxicity when they compared the "methylene blue" as produced by several different manufacturers.<sup>81</sup> Of the five brands he tested, all differed chemically and toxicologically. In addition, he concluded that the minimal lethal dose of chemically pure methylene blue was 41 mg per kilogram of body weight for cats. Nadler thought this figure was high and estimated 7 mg/kg for man and dogs. As cited earlier, others have given much higher concentrations to animals experimentally and rarely reported any untoward reactions.

Attempts have been made to recover or observe methylene blue at different sites in the living organism.<sup>50,92,81,131,109</sup> Deposits have been located in the saliva, gall bladder, stomach, small intestine, urine, feces, and aqueous extracts of muscle tissue when given intravenously or by mouth. Intralymphatically and intravenously it has been used to delineate tumor growths and has been noted to enter the interstitial fluid.<sup>48</sup> Regardless of its route of administration, it is excreted within 24-48 hours via the intestine, kidneys and liver in four forms: methylene blue, methylene azure, and the leuco (reduced) modes of these compounds.<sup>131</sup>

With the above in mind, the adoption of methylene blue chloride appeared to be a reasonable choice for an in vivo pilot study of endodontic filling technics.

#### C. Dogs in Dental Research

The choice of animals as research models is rather extensive. A particular species most likely will be selected according to the experimental design of the proposed project. An in vivo comparison of endodontic filling technics requires teeth not only large enough, but whose root canals are similar in internal anatomy and physiology as those in man. As a research animal in endodontics the dog has produced a long and varied history.

Grove was another one of the adventuresome breed of early dental scientists. Curious about the effects of certain root canal medicaments, he placed different antiseptics in dog root canals before filling them utilizing an "office" routine.<sup>55</sup> He observed a destructive response to the formalin compounds histologically and radiographically. This prompted Grove to vehemently denounce formaldehyde as a therapeutic agent. He found severe inflammatory changes and abscesses forming in the periapical tissues as a result of its use.<sup>54</sup> This takes on a historical interest since this report, plus Grove's earlier published comments, directly opposed the views of another dental giant of that era, Dr. J. P. Buckley. The popular "Buckley Method" of treating gangrenous pulps included the use of a paste made from tricresol and formaldehyde which Buckley had introduced in 1904.<sup>21</sup> In the aftermath, both publically exchanged critical remarks and charges of professional ineptitude in the 1911, 1913 and 1914 journals of Dental Cosmos and Dental Review.

After extirpating the pulps in dog teeth, Coolidge placed fifteen different endodontic medicaments into separate canals for a period of 21 days.<sup>31</sup> Following a histologic study, he announced that all were irritating in some degree to the dog's periapical connective tissues. To avoid improper extrapolation, he qualified his findings by stating: "This

experiment reveals only the reaction to the drugs used on living, healthy connective tissue of dogs in the periapical region of the teeth. Human teeth treated in a similar manner might present a different reaction to the same drugs."

However, Coolidge's work was later interpreted by Orban, his colleague at Chicago College of Dental Surgery, to imply that the periapical tissues of dogs were "more sensitive than human tissues".<sup>97</sup> From this it was postulated that if satisfactory results are obtained following root canal therapy in dogs, then we may accept it as a fair means of evaluation and extend its use to humans.

Working independently, Stein and Hill inoculated living dog root canals with streptococcal organisms.<sup>61,122</sup> They produced granulomas which by roentgenogram and tissue examination were deemed comparable to those in man. Dixon and Rickert assayed the periapical tissues' response to a new sealer in both human patients and dogs.<sup>37</sup> They too concluded that both reactions were identical and that the histologic response in dogs is analogous to humankind. Although with a microscope both look the same, bone tissue following extractions appears to heal faster than that in human beings.<sup>28</sup>

More recently, Barker and Lockett have related a number of valid observations concerning the dog's dentition.<sup>6</sup>

With their evidence, they believe that dog pulp tissue responds to pulp capping procedures with a higher rate of morbidity. This may suggest that canine pulpal tissue is not as hardy as its mankind counterpart. These men also noted that calcific repair did not take place beneath their pulpcaps.

There is a scarcity of literature allocated to the detailed morphology of canine teeth. Even recent veterinary texts devote very little space to this subject. What information that is available is generally concerned with the external anatomy of the teeth. The pulpal tissues and canal configurations are ignored or neglected. Apparently there is no demand for this knowledge in a veterinary practice. Curiously enough, in 1889 "a professorship of dental surgery" was installed at the Chicago Veterinary College.<sup>36</sup> The school gave a course in veterinary dentistry which embraced the anatomy, physiology, and pathology in domestic animals. The demise of veterinary dentistry possible could have been anticipated by the author's remarks when he introduced this course to the dental profession: "We cannot adjust the rubber dam, devitalize the pulp and fill the root as the operator in dentistry can. Even when filling would be practicable we are not given an opportunity to perform the work. Perhaps it would be too much to expect it, when the probabilities are that if the owner has any money to spend on

teeth, it will be on his own." Within his comments quite likely lie the feeling of today's veterinarians too; thus the paucity of dental knowledge in their circles. His concluding statements may well have been an epitaph.

In the current veterinary literature, St. Clair and Jones describe the occlusion and external anatomy of both deciduous and permanent posterior teeth in the dog.<sup>114</sup> Lawson and his colleagues also review the dental anatomy.<sup>77</sup> In addition, they cover the histology of dog enamel, dentin, cementum, pulp and periodontal ligament. In a brief offering, Persson expounds on dental procedures in dogs.<sup>102</sup> Here, he discloses how to extract intact teeth, retained deciduous teeth and fractured teeth. He explains how periodontal treatment may be rendered and a malocclusion relieved through cuspal reduction.

Routine endodontic treatment has been performed on dogs, especially on the cuspid teeth of "working" or sentry dogs. These articles are usually the result of a combined venture by a veterinarian and a dentist.

One team successfully filled a mandibular cuspid using a lateral condensation technic with gutta percha.<sup>78</sup> In 1967, Ramy and Segreto wrote of their success in filling dog cuspids with a mix of zinc oxide-eugenol.<sup>107</sup> In addition they removed the tooth's apex to ensure that all pathologic

tissue was eliminated. Out of ten cases two failed within 3 to 4 months postoperatively. Since their method relies on zinc oxide-eugenol to seal the apicoectomized tooth, it hints that the sealing potential of this cement deteriorates with time. This would be consistent with the observation of other investigators.<sup>35,126,129</sup>

In 1970 Ross and Myers revealed the procedure they have followed in root filling over 200 cases.<sup>111</sup> They injected zinc oxide-eugenol paste into the canals after performing an apicoectomy. Their treatment also required the insertion of a retrograde amalgam. These researchers admit to having no failures while following their cases for 18 months.

In order to be clinically applicable to humans, the instruments and materials should be similarly applied where possible. For this study, mice, rats, hamsters, guinea pigs, rabbits, and cats were quickly ignored because of their wide dental variability and lack of size. Obviously, canal instrumentation on such small creatures would be arbitrary when one keeps in mind the design of available endodontic instruments.

The Hormel or Pitman-Moore strain of miniature swine has recently been proposed for dental research.<sup>134</sup> Their dental development and tissue response have been closely paralleled with that of mankind's. Unfortunately, their initial cost and daily upkeep<sup>y</sup> places them in a economic class



with monkeys, chimpanzees and baboons. Neither can one overlook the arduous task of anesthetizing swine.

The great majority of information concerning dental restorations has come about through their service in extracted teeth. The value of this source of knowledge cannot be overstated. However, a more meaningful evaluation will evolve from their rehearsal in situ. Many practitioners concur with the view that the ultimate input of professional wisdom may be gleaned from their own clinical failures.

The dental and periapical tissues of animals provide a biologic media unequaled by any contrived non-viable model designed to judge endodontic technics. Animal selection for intra-dental experiments, of necessity, places great emphasis on tooth dimensions as well as physiologic applicability. As yet, definitive criteria for animal selection have not been established. Unfortunately the pick of animal does not account for possible differences in tissue responses between species. To eliminate this variable, hopefully a contrast will be made in the near future of the tissue reactions between useable candidates. The same approach, technic, and materials would be employed in each instance, varying only the type of animal. Presently, our animal preference is based on supposition, not scientific fact.

Expanding Tagger's criteria as a basis for choosing the ideal test animal, we may seek the following attributes for endodontic research:<sup>127</sup>

1. Morphogenically the teeth should be similar.
2. The periapical tissues should resemble that of the human anatomically and histologically.
3. The susceptibility to peridental infections should be comparable.
4. The inflammatory response should be applicable and consistent.
5. The animal must not be allergic to the implanted materials.
6. The external and internal tooth dimensions ought to approximate that of humans.
7. Routine dental armamentarium should be adaptable to the animal.
8. The animal must be able to withstand the procedures.
9. The diet must be controllable.
10. The availability, cost and care of the animal should be reasonable.

An overall view of the cited literature would support employment of the dog as a research model. A match-up with the enumerated criteria lend further support, making

them an appropriate prototype for an in vivo comparison of endodontic filling techniques.

## CHAPTER III

### METHODS AND MATERIALS

This project was carried out in two distinct phases:

- 1) Exploring the possibility of evaluating root canal fillings following the in vivo intravenous administration of 1% methylene blue dye.
- 2) Evaluating endodontic materials and technics in vivo with a solution of 1% methylene blue placed intradentally.

A total of 114 root canals in ten dogs were treated to accomplish these experiments. Four dogs and 26 root canals were devoted to the pilot phase of this study where the feasibility of intravenous injections of 1% methylene blue was explored. The remaining six dogs and 88 root canals served as models for the second phase of this project. In the latter phase the same dye was placed in a reservoir above the filling material and sealed within the tooth for a minimum of 48 hours.

The animals selected ranged in weight from 8 to 26 kilograms (Kg.) and were of mixed breed backgrounds. The primary criteria for their selection was that they have a full complement of mandibular and maxillary premolars. The teeth were also examined for freedom from gross caries and coronal fractures. All operations on the animals were

performed in an operating room of the Animal Research Facility at Loyola University Medical Center.

Regardless of the route or method of dye placement, the dogs were all consistently handled in the following manner. General anesthesia was achieved through the intravenous injection of 1 cubic centimeter (cc.) of sodium pentobarbital for each 2.5 kilograms of animal body weight.\* Each animal was weighed immediately preceding every operating session.

Roentgenograms were taken at the same points of progress as customarily taken in a routine clinical endodontic case. This included films to portray the pre-operative condition, establish the working length, and determine the post-operative results. A portable hospital x-ray unit (General Electric-15 Ma) was adapted for dental use by taping a 9" extension cone over the collimator (see Figure 2). It was found that 90 KVP at 10 Ma for 0.5 seconds produced satisfactory films.

Before each scheduled operation, all instruments to be used were completely sterilized. A hot bead sterilizer was ready for use if any other instrument required spontaneous sterilization. In the operating suite masking, gowning and gloving by those participating followed the surgical protocol.

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\* Holmes Serum Co. Inc., Springfield, Ill.

Jaw retraction was achieved with a self-retaining, spring-type retractor. A rubber dam was applied to appropriate teeth in the arches and stabilized with rubber dam clamps. The teeth to be operated on were also ligated with heavy waxed dental floss to aid in gingival retraction and control local hemorrhage where it occurred. The dam surface and exposed teeth were then swabbed with a 70% alcohol solution.

A standardized method of preparing each tooth evolved through preliminary trials. The occlusal surface was reduced with diamond or heatless stones to a flat horizontal plane for reference measurements. This not only completely reduced the tooth out of occlusion but also exposed the central pulp horn which guided the unroofing of the pulp chamber. A path 1 mm. in depth was cut into this flattened surface with a fissure bur producing an occlusal (Class I) preparation resembling that prepared in a human posterior tooth. The remainder of the access preparation was completed with a # 4 round bur to expose the chamber completely and create undercuts for the future temporary cement.

The mesial and distal canal orifices could now be readily located with an endodontic explorer. Each canal was first instrumented with a # 30-35 file to initially flare the orifices of the root canals which persistently were found to be constricted. This aided in broaching the pulpal contents

and later instrumentation within the canal. Endodontic files (# 25), with rubber stops set flush with the flat occlusal surface, were seated to the depth of each canal to determine its length radiographically. While this roentgenogram was being processed the canal contents were further broached of all soft tissue. With the "working length" established, the canals were totally cleansed and shaped according to the type of root canal filling they were to receive. An attempt was made to simulate the clinical preparation common with each technic; i.e., for the gutta percha procedures a funnel-shaped preparation was made by lateral filing action plus the "step" method of canal instrumentation. The preparation for silver cones was made by a direct reaming action. Throughout the preparation procedure the canals were generously irrigated with a sodium hypochlorite solution. Before filling, each canal was thoroughly dried with cotton pellets and paper points.

Separate canals were filled with the following materials:

- 1) segmented gutta percha cones plus chloropercha solution.\*
- 2) segmented gutta percha cones plus Kloroperka N-O solution.\*\*

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\* Thin solution of gutta percha cones dissolved in chloroform.

\*\* Union Broach Co., Inc., Long Island City, N. Y.

- 3) lateral condensation of gutta percha cones with varied consistencies of Proco-Sol radiopaque silver cement.\*
- 4) silver cones with Proco-Sol radiopaque silver cement.
- 5) thick Proco-Sol radiopaque silver cement alone.
- 6) thin Proco-Sol radiopaque silver cement alone.

The access opening was finally sealed with zinc oxide-eugenol cement. In all, each fill session lasted approximately five hours from the time of anesthetic injection until the temporary fillings were inserted.

Another facet of this project was to evaluate chloropercha and Kloroperka N-O root canal fillings and technics performed by an individual who was experienced in their manipulation. If an evaluation of materials is to be valid the material must be placed in a consistent fashion and by an operator competent with its use.<sup>68</sup> Dr. Henry Kahn of the Loyola University School of Dentistry's endodontic department has routinely utilized these materials for forty years in his own dental practice. He gained the technic as a student and young practitioner from Loyola's Dr. Edgar D. Coolidge.<sup>33</sup> Dr. Kahn placed all of the chloropercha and Kloroperka root canal fillings tested in this study.

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\* Proco-Sol Chemical Co., Inc., Philadelphia, Pa.



Dr. Kahn's technic is to adapt the apical end of a non-standardized gutta percha cone to the working depth of the prepared canal. Approximately three millimeters of this cone's tip is heat-tacked onto the end of a suitable long-handled Luk's plugger.\* This gutta percha segment is dipped into the chloropercha or Kloroperka solution for five seconds which softens the outer cone surface while a small amount of the solution clings to it as well. The initial segment is seated to the working distance in the fully dried canal. Apical pressure is maintained with the plugger while it is rotated, locking the gutta percha into place. This motion also frees the segment from the plugger. Smaller diameter Luk's pluggers are then utilized to vertically condense this initial segment of gutta percha. Additional 3 mm. pieces of gutta percha are dipped in the particular solution and similarly added as before until the full length and breadth of the canal is filled.

A method of surgical extraction was devised to recover the premolar teeth intact (see Figure 1). An "envelope" type of mucogingival flap is retracted, assisted by relieving incisions anteriorly and posteriorly. This tissue flap extended to an additional tooth on either side

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\* Union Broach Co., Inc., Long Island City, N. Y.

of the teeth to be removed. The mesial segment of the tooth is separated from its distal member by cutting through the furca in a buccal-to-lingual direction with a # 560 fissure bur. This cut is continued inferiorly, bisecting the angle between the two roots to about their suspected apical level. This channel is extended buccally and lingually completely through both cortical plates. A diastema normally exists between the mandibular premolars. Other vertical channels are cut through this interdental space. These grooves serve a twofold function. They reduce the "grip" on the tooth by one entire wall and also provide convenient purchase points. Now, a periosteal elevator, bone chisel, or broad-tipped elevator (i.e. # 34) may be used to lever the buccal cortical bone segments from between the perpendicular channels. A gentle rocking motion is used to luxate the individual roots with the # 34 elevator. The use of forceps alone is not advised since very little rotational movement is normally possible. The path of exit must be in the long axis of the roots. Immediately following the extractions the animals were sacrificed, while still anesthetized, using Totaltox.\*

After extraction, the teeth were again x-rayed and photographed for permanent recording of their appearance.

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\* Chicago Veterinary Supply Co., Chicago, Illinois

The root portions from Pilot dogs # 1 and 3 (I.D. # 713 and # 143) were then sectioned serially. These ground sections were cut 1 mm. thick at the American Dental Association optical research laboratory. These discs were studied under a dissecting microscope at 20 to 40 X magnification. Any discoloration at each 1 mm. level was noted. This technic was found to be not only laborious but the dye stain was very difficult to discern at the root canal filling/tooth junction. Beginning with pilot dog #4 (I.D. # 163) and continuing through the remainder of the study, the teeth were sectioned vertically through the root canal so that the full extent of the canal wall and filling material could be seen unencumbered (described later).

#### A. Phase I Technics - Intravenous Dye

Four dogs were used for preliminary studies.

##### Pilot Dog # 1 (I.D. # 713)\*:

Under general anesthesia, trial endodontic fillings were manipulated into the mandibular 2nd, 3rd, and 4th premolars and the mandibular left 1st molar. A temporary cement of zinc oxide-eugenol was inserted into the access preparations once the canals were filled. The right mandibular 3rd and 4th premolars were splinted tightly together with # 20 gauge stainless steel wire. The purpose of the wire was to produce periapical inflammation and hopefully induce the dye to escape from the capillaries of the periodontal ligament space.<sup>8,48</sup>

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\* Animal identification number

A solution of 1% Methylene Blue Injection, U.S.P.\* was given in a concentration of 20 mg/kg. The dye was further diluted to 0.25% in normal saline which had been previously warmed to body temperature. A total of 180 mg. of dye was administered by the intravenous (I.V.) drip method. A buretrol I.V. unit\*\* was used to control the dilutions and perfusion rate for all intravenous studies. The perfusion took approximately 90 minutes. According to other investigators, the pre-warming, the dilution and prolonged injection would substantially reduce the possibility of any toxic reaction due to the elevated concentration levels (1-2 mg./kg. is the usual therapeutic dose).<sup>81,124</sup>

The animal mysteriously expired approximately 2 hours after beginning the infusion. One maxillary premolar was extracted shortly thereafter to determine if any dye had reached the alveolus. Since no trace of stain was seen on the cementum surface, holes were drilled into the cancellous bone just beyond the apices of the right maxillary and mandibular premolars. Undiluted 1% methylene blue was deposited into these holes for 24 hours, after which the maxillary premolars and trial-filled teeth were extracted.

#### Pilot Dog # 2

A trial injection of 20 mg./kg. methylene blue was given intravenously to a 10 kilogram dog. The concentration was diluted 1:4 with sterile saline and controlled in the Buretrol intravenous unit. The animal began to show signs of angioneurotic edema after approximately 25 milliliters (125 mg.) had been infused. The intravenous unit was stopped and the dog returned to his cage.

#### Pilot Dog # 3 (I.D. # 143)

A trial injection of 10 mg./kg. was given to a 15 kilogram mongrel dog without invoking toxic effects. At a later date, endodontic fillings were inserted in the mandibular premolars. The animal then received 3 cycles of the dye intravenously at a concentration of 10 mg./kg. of body weight, diluted to 0.25% with injectable saline. These injections were given 48-72

\* American Quinine Co., Inc., Plainview, N. Y.

\*\*Travenol Lab., Inc., Morton Grove, Illinois

hours apart. The average length of time to inject the entire 150 mg. of dye was 68 minutes. Thirty minutes after the third cycle of dye injection was complete, an unfilled tooth was removed and its root surface examined for traces of dye. Since no clinically visible evidence of the dye was observed on the tooth, holes were drilled into the cancellous bone just beyond the apices of the root-filled premolars. The 1% dye was injected directly into these channels and allowed to remain for 4 hours. The excess methylene blue was then removed from the holes with cotton pellets to avoid its coloring the teeth during the extraction process. Serial horizontal ground sections were made of the filled roots.

Pilot Dog # 4 (I.D. # 163)

Following an uneventful test injection of 10 mg./kg. in an 18 kilogram dog, the mandibular premolars were filled using a lateral condensation technic with gutta percha. Two weeks later a total of 18 milliliters of 1% methylene blue diluted 1:4 was injected as before over a span of 57 minutes. Soon afterward, allergic manifestations developed similar to the # 2 pilot animal. A tissue sample was taken from the right maxillary cuspid area gingiva following the infusion to check for the gross presence of dye histologically.

One week later the abdominal cavity was opened and both kidneys were dissected out by a veterinary surgeon. The renal arteries were ligated with 6.0 silk near their entry into the fundus of the kidney. The abdominal incisions were then closed in layers. Immediately afterward, a total of 36 cc. of the 1% methylene blue (20 mg./kg.) was added to 36 cc. of normal saline. This solution was injected via the external jugular vein because the extremity veins usually used were not only difficult to enter, but tended to collapse over the intracath. This injection took 53 minutes once the external jugular vein was entered. Four hours after starting the perfusion an intact pulp and another sample of gingival tissue were excised for biopsy.

Again, no dye was seen on the roots following a test extraction. Holes were then created into the underlying spongy bone beyond the apices of the treated teeth on the right side and at the apex of

the left mandibular 2nd premolar. The holes were saturated with the 1% dye for an additional hour before the teeth were removed.

Thus, these teeth could potentially receive the dye in at least 2 and in some cases 3 routes:

- 1) I.V. - 10 mg./kg. 2 weeks prior to extraction.
- 2) I.V. - 20 mg./kg. for 4 hours with renal arteries tied.
- 3) directly from a subjacent bony reservoir.

These teeth were sectioned longitudinally and opened by fracturing them through the canal for complete visualization.

#### B. Phase II Technics - Intradental Dye

A total of 88 root canal fillings from six dogs were evaluated. As in phase I, technics and materials were handled as they would be clinically.

Where silver points were to be employed, all canals were prepared to a # 70 or # 80 file to the depth of the canal. This was necessary because the internal anatomy of the dog premolar root canal is somewhat "cigar-shaped". The apex of the canal is blunt-ended rather than tapered as usually found in humans. In order to produce a reasonable silver cone preparation in such a canal, instrumentation to larger sizes was indicated.

All of the plastic type root canal fillings were trimmed of excess filling material leaving about 7 mm. of filling substance in the root canal. A crypt was created in the coronal portion of the canal and chamber by warm instruments and/or small round burs where the dye could be housed.

Gross heating of the instruments at this time was shunned in an effort to minimize thermal strains from being imparted to the materials as warned by Price and McElroy.<sup>106,85</sup> The occlusal access openings were then sealed with zinc oxide-eugenol cement for one week, allowing the root canal fillings to mature. As a precautionary measure, all test animals were sustained on a softened food diet to avoid tooth fractures and dislodgement of the temporary occlusal seals for the duration of the test period.

This project also considered canals filled with thick and thin mixes of Proco-Sol radiopaque silver cement. Not only could this popular cement's faculty as a sealant be tested but also the effectiveness by which it usually is manipulated into a canal be observed.

A "thick" mix of Proco-Sol was produced when two capsules of powder were thoroughly spatulated with two drops of liquid. A small amount (approximately a half of one drop) of the Proco-Sol catalyst was then added and mixed until all evidence of graininess was removed. This mix would string one-half inch before breaking.

A "thin" mix of the same cement was composed of two capsules of powder plus two drops each of the liquid and catalyst. These proportions resulted in a mass which would string for one inch before breaking.

Both mixes were introduced into canals prepared as though to receive gutta percha root canal fillings. These canals were instrumented to a # 40 file at the apex and funneled to at least a # 70 file at the coronal end. Both thicknesses of sealers were usually spun into the canal preparations with a hand-held Lentulo type paste filler\* turned clockwise. Thus, the only variable introduced was the consistency of the sealer.

A consistent plan was adhered to for depositing the 1% methylene blue dye and assure absolute contact with the restorative substance. After allowing the endodontic materials to cure for one week, the temporary occlusal seal was entirely removed. The extent of the chamber and canals was re-assessed to ensure that an ample crypt existed for the dye. The chamber was flushed with a sodium hypochlorite solution to moisten all surfaces and the excess aspirated. The methylene blue was deposited directly on top of the root canal filling by a tuberculin syringe with a # 25 gauge needle. Then the dye was repeatedly injected and aspirated with the syringe and the chamber inspected again to see that the testing solution completely wetted all surfaces within the tooth. A small cotton pellet was introduced into the

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\* Union Broach Co., Inc., Long Island City, N. Y.



crypt to "hold" the dye. Before sealing the occlusal access again with zinc oxide-eugenol, the cotton pellet was re-saturated with more dye. Two days later these teeth were surgically removed, then radiographed and photographed again.

The only departure from this routine was in one case (I.D. # 298) where the dye was placed on the same day that the canals were filled. This allowed a comparison of "new" versus "aged" root canal fillings.

After the photographic records were taken, the roots were ready for sectioning. Aided by Loupes, the coronal end of the root was carefully removed with a high-speed handpiece and crosscut fissure bur to the level where the restorative material filled the prepared canal throughout its breadth. The occlusal's periphery was then beveled leaving a narrow ring of dentin around the filling material. A narrow, vertical groove was cut with a #  $\frac{1}{2}$  round bur along the entire length of the remaining root portion. A second groove was similarly cut  $180^{\circ}$  around from the first groove. Both vertical grooves were cautiously made so as to avert perforating the underlying root canal wall. The final layer of dentin was fractured by fitting these longitudinal grooves onto the leading edge of side-cutting Rongeur forceps. The entire root canal filling, root canal preparation, and any dye penetration could now be completely examined. As

the root canal filling material was removed its characteristics and appearance were also noted.

Penetration was "read" by measuring the greatest length of linear invasion by the dye. Any bluish discoloration on the canal wall was interpreted as being due to a discrepancy between the filling matter and the prepared dentin surface. The extent of dye penetration was measured with a Boley-Guage micrometer, aided by Loupe's magnification. Each measurement was taken at least twice. If successive readings differed by more than 0.3 mm., it was re-measured at a later time.

## CHAPTER IV

### RESULTS

#### A. Phase I Findings - Intravenous Dye

The results of the intravenous phase of this study are as follows:

##### Pilot Dog # 1 (I.D. # 713)

A necropsy report stated that likely causes of death included congestive pulmonary edema, heart failure, or anesthetic overdose.

The maxillary left 4th premolar was extracted 2 hours after beginning the dye infusion. Gross observation revealed little, if any, trace of dye on the root surface. Somewhat more dye was observed on the roots situated close to the bony dye reservoirs, however, it was hardly enough to be of value. Serialized ground sections observed under low magnification confirmed the gross observations, that is, only light traces of coloration were seen on the cementum surface. No coloring was seen around the filling materials within the canals (see Figure 3).

Treating two teeth with hydrogen peroxide in order to test for the presence of leuco-methylene blue gave negative results.<sup>131</sup>

##### Pilot Dog # 2

This dog was deleted from the study because of its untoward response to the dye.

##### Pilot Dog # 3 (I.D. # 143)

Very light traces of dye were seen on the root surfaces. No dye was visible within the confines of the root itself.

## Pilot Dog # 4 (I.D. # 163)

The right mandibular 3rd premolar shattered during the extraction process and was eliminated from the study. A pre-existing ankylosis was suspected because no lateral movement was gained through the use of broad bladed straight elevators.

Visual examination of the root surfaces revealed no evidence of blue coloration on any of the treated teeth. The entire cementum surface of both mandibular 2nd premolars (whose apices were near the dye reservoir holes) was lightly coated with the dye.

No evidence of the dye was clinically obvious upon vertical sectioning of the roots (see Figure 4).

The histologic search for the gross presence of methylene blue in the gingiva and pulp tissue was negative.

In all dogs comprising the intravenous phase, the saliva assumed a distinct blue-green hue within thirty minutes after initiating the dye flow. Also, within that length of time the entire tongue and oral mucosa became bluish-purple in color. This coloration was not perceptible after 24 hours.

#### B. Phase II Findings - Intradental Dye

When viewed individually, the results appear to be consistently inconsistent. This is especially remarkable since the treated premolars were all anatomically uniform in configuration, size, and number. In addition, the same operator accomplished every filling in each grouping and each group of root canal fillings was manipulated by the same individual at a single sitting in one animal. Both operators would be considered as completely familiar with

the materials and technic each wielded. It is reassuring to realize that others have confessed an equivalent diary.<sup>47,112,113</sup> In this same vein Harper poignantly remarked that, "the inconsistency of the results has been a revelation and mortification to me".<sup>58</sup>

The degree of penetration varied from tooth to tooth and from root canal filling to root canal filling. If anything, our measurements could be generous to a fault. In some cases the qualified discoloration was so slight that its actual presence was suspect. The prevailing attitude was that regardless of the intensity of the dentin's foreign coloration, any bluish discoloration would represent penetration. Intensity is difficult to convert to a numerical value because the judgment itself is a subjective process. The estimated value when plugged into a graph or table hopefully yields an unbiased, relatable message (see Tables 9 and 10).

In all cases where the root canal was fully prepared to receive a root canal filling but was left unfilled, the dye penetrated to the apex. As it was applied in Phase II of this project, 1% methylene blue is a reliable test agent. The method used to apply the dye was equally reliable. This dye was not seen penetrating into the mass of any of the bulk filling materials studied in this thesis, however, it

does penetrate an estimated thickness of 1.5 mm. of the Proco-Sol sealer. Our preliminary work with both human and dog teeth as well as glass capillary tubes showed that methylene blue will permeate Proco-Sol sealer to an approximate depth of 1.5mm.

On the day the dye was sealed within the dog's teeth some of it was incorporated into the mass of the occlusal zinc oxide-eugenol temporary cement turning it bright blue in color. After 48 hours, the color was no longer visible. The reason for the disappearance of this coloring from the temporary access sealant is not understood at this point, but the color appeared to transfer from the zinc oxide-eugenol cement into the dentin and enamel surrounding the occlusal access preparation. Coronally, the methylene blue dye pervades the entire dentin and enamel fabric staining it a brilliant blue. The external pattern of coloration is not a precise indication of the internal distribution of methylene blue. The dye will extend further along the internal dentin surfaces than is apparent when the tooth is scrutinized externally. This may be due in part to the distribution and histology of canine dentin tubules.

When the dye penetrates the margins of a root canal filling, it does so unevenly. We generally see irregular projections of discoloration along the root canal wall

resembling pseudopodia. Also, the intensity of this staining is not usually uniform around  $360^{\circ}$  at any level of the root canal wall. When we see a band of blue color on this inner dentin wall projecting apically, it represents a void in the margin only at that site. That void may be due to operator failure or it may be indicative of innate inadequacies in the physical properties of the endodontic material itself. Our preliminary studies indicated that a 48 hour period gave sufficient time to reveal any marginal discrepancies.

# 166 - Segmented Gutta Percha Cones with Kloroperka N-O:

One control tooth was lost during the extraction process. The remaining three controls contained no root canal filling materials. The canal wall became intensely stained from crown to apex. The dye also permeated the dentin in toto.

The gutta percha with the Kloroperka solution acting as a sealer produced a smooth, homogenous mass as a root canal filling material. This mass does not appear to form a complete peripheral bond with the inner dentin wall. It adheres to the prepared root canal wall in small patches only. The primary material tended to be brittle and only three root canal fillings could be removed intact. The path of the dye was evident on both the dentinal wall and surface of the material itself. The intensity of dentin discoloration

was "heavy" when assaying this aspect. The intensity was greatest coronally and gradually decreased toward the apex (see Figure 8).

While filling the distal root of the right mandibular third premolar, a void was observed according to a radiograph. The root canal filling was left unaltered to observe whether the methylene blue could reach that level. The depth of penetration was 3.8 mm. in one half of this root canal wall while measuring only 1.9 mm. in its other half section (see Table 1). Dye diffusion for the Kloroperka method was the severest of all the substances and technics included in this study, averaging 4.14 mm. penetration per root canal filling.

The mesial root of the right mandibular premolar was omitted from consideration as the dye appeared to have been improperly placed.

#166 - Proco-Sol (thick):

The dentin is stained "moderately heavy" in general around the entire perimeter of the thickened sealer. The intensity of dentin discoloration is remarkably uniform at the uppermost level. The fade-out of this coloration ends quite abruptly as one follows it toward the apex (see Figure 9). The average depth of dye seepage was 1.77 mm. here.

In the mesial root of the left maxillary third bicuspid, a crater (depression) or a tiny hole persisted after



removing the Lentulo during the root canal filling procedure. If the crater or hole remained while the sealer hardened, the dye could be expected to explore the defect. This appeared to be the case as this root canal exhibited the greatest linear penetration of the seven evaluated in this group.

# 298 - Segmented Gutta Percha Cones with Chloropercha:

In this animal the dye was sealed over the root canal filling immediately after the obturations were completed. The control teeth (prepared but not root canal filled) were darkly stained the entire length of the preparation. The dye fully permeated the dentin also. The filling material is a homogenous mass but fractures readily. Where dye stain occurred in the marginal dentin it is "very light" in general. Its mean linear penetration was 1.49 mm. The brilliance of the discoloration fades somewhat rapidly toward the apex (see Figure 10).

# 301 - Segmented Gutta Percha Cones with Chloropercha:

The dye here was placed one week after the teeth had been treated and root canal filled. The mandibular right second premolar roots were initially intended for controls but were fractured by the dog on his cage bars, and had to be deleted. Without any endodontic material in the mandibular left second premolar (controls), the dye diffused unimpeded

the full length of the root canals. Compared to the Kloroperka sealer, the chloropercha did not cling to the walls nearly as well.

The physical consistency of these root canal fillings appears to be homogenous in nature. Their surface is very smooth indicating that the prepared root canal walls were well-planed. Although the filling masses were somewhat brittle, four of them could be retrieved from the root canals. "Moderately heavy" stained dentin was observed around the entire circumference of the root canal filling. After being allowed to age for one week, there was more than 200% deeper penetration than that seen when the testing solution was placed on the day of the root canal filling (1.49 mm. vs. 3.63 mm.).

For the matured chloropercha the greatest amount of marginal diffusion was observed in both roots of the right mandibular fourth premolar (see Figure 11). On the day of operation, a notation was made that a great deal of hemorrhage exuded from this tooth. Success in the chloropercha and Kloroperka technics relies on absolutely dry dentinal walls. The presence of moisture acts as a physical deterrent, not allowing these materials to intimately contact the dentinal surface.

# 301 - Proco-Sol (thin):

When sectioning these teeth, this root canal sealer was extremely brittle and did not adhere well to the dentin. This material tended to shatter while the teeth were being fractured with the Rongeurs. Therefore, hand instruments were utilized in place of the Rongeurs to eliminate the crushing force and retain as much of the root canal sealer as possible in its applied position.

Multiple voids existed in the body of the sealer mass although a conscientious effort had been made to fill the canals in their entirety. Only one canal (distal, right maxillary second premolar), was judged to be well filled while two others were thought to be fairly well filled by this sealer alone when examined immediately after sectioning.

The dye was "light" in intensity at the occlusal 1 mm. level then quickly paled toward the apex (see Figure 12). In only two of the eight fills was the dentin penetration considered "heavy". The comparative depth of dye penetration for similarly inserted thick or thin consistencies of Proco-Sol sealer is notably close (1.77 mm. vs. 1.79 mm.).

## # 322 - Gutta Percha Cones with Proco-Sol (thin):

Here, control canals were prepared to a # 50 file at the apex and funneled to at least a size # 70 file at their orifice. A handpiece-driven Lentulo paste filler spun the

thin mixture (as recommended by the manufacture) into the four root canals using them as controls. The large size of the preparation allowed the Lentulo to freely deposit the cement without binding. It is noteworthy that these four controls produced the least linear penetration by the methylene blue (1.25 mm.) in this investigation. The "light" dentin coloration existed completely around the prepared walls and ended abruptly with very little fading (see Figure 13).

The lateral condensation technique employed here resulted in a homogenous mass of gutta percha which appeared to be well adapted to the smooth walls of these preparations. Evidence of dye was seen on the gutta percha surface in five of the six fillings placed in this animal. The dye was especially visible in roots where the longest penetrations also took place. In the one case where the dye was not seen on the gutta percha surface, the linear penetration was but 1.2 mm., compared to the overall group mean of 3.65 mm. The mesial roots of both third premolars were eliminated from evaluation when the animal fractured them prior to their extraction. In three of the remaining roots, the longest extent of discoloration was so pale that their actual penetration was reasonably doubtful. Therefore the average length of linear penetration here is deceptively excessive.

# 326 - Silver Cones with Proco-Sol (thick):

The root canals used as controls were prepared to a size # 60 file, while those to receive test root canal fillings were instrumented to a # 70 or # 80 at their apex. Two of the controls were fitted with silver cones and wedged into place without sealer. Heavy deposits of dye were seen around the entire circumference of this prepared surface. The remaining two control canals were filled with thick Proco-Sol radiopaque root sealer by turning a root canal file counter-clockwise. Large voids were seen in both controls filled with the thick sealer. In this manner, the relatively deep penetration was quite likely due to poor manipulation and flow characteristics rather than due to the material's lack of sealability.

Although a ring of stain was generally observed here, the intensity of discoloration was "very light". Discoloration was noted on the surface of four of the eight silver points. In restorations where the dye appeared on the metal's surface, there was considerable linear penetration also (note the distal root of the mandibular right third premolar and both roots of the third premolar plus the distal root of the fourth premolar on the left side). It is interesting to observe that the average straight-line penetration for these four was 3.43 mm. whereas the average for

the remaining four where the dye was not evident on the silver point surface averaged 1.15 mm., or three times the length of discoloration. This finding was similar to that seen in the case # 322 with laterally condensed gutta percha with root canal sealer. There, five of the six laterally condensed gutta percha masses had dye on their surface. Their average marginal penetration being 4.16 mm. whereas the sixth gutta percha filling exhibits merely a 1.2 mm. depth of dye diffusion (see Figures 13 and 14).

Small voids were observed in the film of sealer surrounding the silver cones. This may be the same "laking" which Weiner has reported from his in vitro studies of endodontic sealers.<sup>138</sup> In the present study, silver cones cemented with Proco-Sol gave the best overall results of those methods commonly used in endodontic practices today.

# 331 - Gutta Percha Cones with Proco-Sol (thick):

Two controls were filled with this consistency of sealer by using root canal files. After sectioning, it could be seen that both canals were incompletely filled. The linear penetration in one was 1.6 mm. while the other was 5.6 mm. One reasonable explanation for their broad difference in penetration is that the thicker mix seemed to display poorer flow properties. A single gutta percha cone was adapted and seated into the remaining two control canals.

The methylene blue readily stained the full extent of the prepared canal in each instance (see Figure 15).

Unfortunately, the bulk filling materials from this aspect of the study were discarded. It would have been interesting to search their surface for traces of dyes and compare the findings with those of animals # 322 and # 326. It is rational to speculate that the higher average of penetration seen here (3.95 mm.) may also represent some diffusion through the sealer and onto the dentin wall by dye that crept between the gutta percha/sealer interface.

Of interest here was the observance that the intensity of the dentinal staining was interpreted as either "very light" (in 2 test teeth) or "heavy" (in the remaining 6 trials) and always encircled the entire canal. Although the two were noted to be "very light", they averaged 5.15 mm. of linear penetration whereas the six decreed to be "heavy" averaged 3.55 mm. or 1.50 mm. less! This observation agrees with the gutta percha root canal fillings laterally condensed in animal #322. There, 6 trials were "light" and averaged 4.55 mm. in linear diffusion while the 2 classed as "very heavy" bore a 3.20 mm. linear average of dye. In the latter animal, "moderately heavy" deposits of dye extended 1.35 mm. less than when the root canal filling was "lightly" penetrated.

With the silver cone, chloropercha, and Kloroperka experimental root canal fillings the heaviest dispersion of methylene blue dye corresponded with the deepest linear extent of penetration.



## CHAPTER V

### DISCUSSION

#### A. General Observations Involving this Study

Through the years a multitude of tests have been employed to assess the marginal integrity of restorative materials used in dentistry. Vast amounts of scientific knowledge have been made available allowing the profession to rapidly progress. More directly, scientific research has helped to enhance the public's confidence in dentistry while raising the level of professionalism. The experimental methods used have had varying degrees of merit.

Because of expediency, time, and availability, many tests have been performed primarily on extracted human teeth. When testing the margins of root canal fillings, additional problems must be confronted and solved. The ultimate and only valid test of any root canal filling (including substances used, operator ability, technic, etc.) must take place in an acceptable biologic model.

Many aspects of endodontics have been investigated through the use of laboratory animals. These generally involved the assessment of how a particular material and/or medicament effected or affected tissues. The prime concern

was, "how will the tissues be affected if that substance invades them during the course of treatment or soon thereafter?".

The bane of the endodontist has been root canal failure and how to avoid it. Hence, the constant search for new materials and improved methods which will help us seal the root canal system from the body. When performing the root canal filling procedure we can not be sure if we have sealed off all the internal pulp channels despite our best radiographic evaluations and clinical acumen.

To test the sealing ability of root canal fillings, ingenious experimental designs have been implemented. Many test solutions have been pitted against the margins of root canal fillings including dyes, inks, bacteria, isotopes, and fluorescent substances. But, all have been applied to the non-functioning, non-living tooth! We now must know the fate of these substances and procedures in vivo. Such information is analogous to the terms "statistically significant" and "clinically significant". Certainly the knowledge gained from the laboratory may be statistically significant - in the laboratory situation, yet may be found to be clinically insignificant.

In the 1930's while studying tooth metabolism by means of "dental lymph" Bodecker and Lefkowitz were among

the first to point out that dentin permeability differs in vital and pulpless teeth.<sup>14</sup> They demonstrated that a dye placed in an empty root canal will exhibit greater diffusion than in one where the pulp is present. Also, this penetration will differ in sound and old (sclerotic) teeth. Loisel and his colleagues found that in vitro hamster teeth exhibited greater marginal permeability when amalgam restorations were compared under in vivo and in vitro conditions.<sup>79</sup> Going discovered that temporary stopping placed in vitro teeth provided a good initial barrier but when he repeated the experiment in vivo its penetrability increased sixfold.<sup>47</sup>

Going also has emphasized that the same restorative materials tested in the laboratory gave different results when similarly tested in a living organism. It becomes evident that we can no longer ignore the difference in response of the same dental materials under morbid and vital conditions. Furthermore, we are rapidly exhausting the variety of tests to which we can subject teeth in the laboratory, therefore we must explore means of evaluating our endodontic treatment aspects within viable tissue. Failure and loss of the tooth from the mouth becomes "clinically significant" regardless of whether bench tests of a procedure are "statistically significant" or not.

The possibility of an intravenously placed dye reaching the dental tissues seemed remote, yet had intriguing

potential. The location of tumorous tissues with vital dyes is well known. A number of dyes have been employed to accomplish this, including methylene blue. Some authors stated that the I.V. dye would reach the interstitial tissues.<sup>48,49,50</sup> Therefore because of its contrasting color, non-toxicity, documented broad previous use, economy, and availability, methylene blue was chosen.

The decision of injecting the dye on three separate days 48 to 72 hours apart was made with full knowledge that the dye was normally eliminated in 24 hours. Actually, the elimination begins soon after entry of the dye into the body. If it could reach the interstitial spaces its concentration would be highly dilute. Therefore, a supposition was made that a three-cycle - perfusion would permit adequate exposure and accumulation. To determine if renal excretion of methylene blue prevailed, the kidneys were excluded from the circulation in one pilot dog since it is one of the major organs for the dye's elimination. These proposed measures proved to be of no avail.

The teeth were carefully examined for the dye's presence following the injections. Little, if any, was apparent. Because of its oxidation-reduction capacity, a portion of the dye in circulation is said to exist in the leuco - or "invisible" (oxidized) form. To rule out the

possibility that the dye was indeed present, but not in its original form, some extracted teeth were placed in an oxidizing solution to reduce any unseen dye back to its visible (reduced) form.<sup>131</sup> This act not only removed the precious traces of dye present and evident on the root surface, but produced a well bleached tooth!

For the sake of consistency the dye was always injected intravenously. The possibility exists that an injection into the carotid artery may have achieved our goal as Spector avers that the arterial end of the capillary is more permeable.<sup>121</sup> Drugs could also have been given to increase the capillary permeability. In the present project, a localized increase in capillary permeability was attempted by the production of a mild localized inflammation induced by tightly splinting two adjacent teeth together. This step did not achieve the goal of attracting additional dye to the area. Perhaps the injury must be more severe to be effective for this purpose.

Except for the mandibular second premolars in dog # 163, the only traces of dye observed were on the apical one-fourth of the roots (see Figure 4). Whether these deposits represented true diffusion (dye was also placed close to their apices) or if the dye was picked up in the extraction process cannot be positively confirmed. Regardless, the amount was

insufficient to act as a guage for in vivo apical penetration studies.

In animal experiments regarding endodontics a number of preliminary questions must be answered: 1) Are the chosen animals and human teeth comparable? 2) What is the morphology of the teeth and the root canal? 3) Are the canals amenable to reasonable preparation and restoration with available armamentarium? 4) Do these teeth respond comparably to the testing agent? 5) Is the agent compatible with life when used intravenously? 6) Is the intravenous route feasible or could the agent be more opportune if used locally? 7) How much of the agent must be injected to get a "reasonable" reading? 8) What is the optimal time period between injection, dispersion and animal sacrifice for that agent? 9) What physical condition will the animal be in while dye dispersion takes place? 10) Does the agent have an affinity for other structures as well? 11) What is the in vivo permeability of the outer tooth structure to the agent used?

It becomes readily apparent to anyone so inclined, that an in vivo study requires a great deal of cerebation in order to come up with a reasonable experimental design.

While perfusing the animals with 1% methylene blue injectable, U.S.P., angioneurotic edema occurred in three

different dogs, a circumstance which to our knowledge has not previously been reported (see Figure 6). After the first episode, the dogs used in Phase I were given trial injections intravenously and observed for a reasonable length of time before commencing with the endodontic treatments. Unfortunately this technique offers no absolute assurance of immunity. In pilot dog # 163 these same symptoms did not develop with the trial injection, yet were manifest with a subsequent injection. Ironically these reactions may have been due to the fact we were using a chemically pure and sterile form of methylene blue. Macht concluded that the more refined this dye is, the more potentially toxic it becomes.<sup>81</sup> In Conn it is stated that a "pure" form must be used for vital staining, however, the pure form is a poor histologic stain.<sup>30</sup> If micrometric quantities of the dye reached the periodontium, methylene blue lacked the gross staining capability needed to act as a detectable indicator for the evaluation of endodontic procedures.

Another possible explanation of the toxic incidents and the peripheral vascular collapse we experienced, may have been due to not using injectable glucose as a physiological dilutant for the dye (see Figure 7). Brooks felt this was not necessary because she believed that a healthy body's natural glucose content was adequate. Glucose apparently

acts as the catalytic agent in the redox cycle which re-converts methemoglobin ( $\text{Fe}^{+++}$ ) back to hemoglobin ( $\text{Fe}^{++}$ ), thereby avoiding an accumulation of the methemoglobin. In addition, many investigators (cited earlier) injected this dye intravenously without any specified dilution or precautions in most cases - even when concentrations of 100-200 mg./kg. were injected. Some have mentioned the peripheral collapse, but this was not considered to be a major deterrent or contraindication for its use.<sup>50,81</sup>

There is a need for in vivo research with dyes. Their employment offers considerable promise as a dental detective agent. The medical, biological, toxicological and chemical literature carry much pertinent information. While in reality there is very little known concerning dye toxicity, elimination routes, target organs, and natural tissue barriers. Also, it should be noted that dyes not readily available in the U.S.A. are being used in other countries to demonstrate the location of cancers. In vivo dyes may prove to be advantageous in future dental research as well.

Placing a dye within a tooth is a legitimate test of the sealing capability of a dental material. Here, the entire filling/tooth junction is subject to the dye for penetration. This magnifies the sealing ability (or lack of it) because a large marginal surface is exposed to the dye.



Unless a canal is prepared completely through the apex, tests of root canal filling materials against apical penetration cannot be considered valid. Where the root canal preparation is short of the apex, the apex becomes inadvertently packed with dentin filings in most instances which will inhibit penetration.<sup>3</sup> Also, the entire margin is not exposed to the testing agent, therefore, a portion of the "seal" is provided from the surrounding cementum, dentin, or predentin. Having a closed or narrow apex provides an excellent apical stop and is not wholly a test of the root canal filling material's ability to seal the root. We can see how an evaluation of a material which is vertically condensed against a closed apex will inadvertantly embrace the ability of the technic rather than the material to seal a cavity in the root.

A popular test method is to fill the root canals of anterior teeth, then cover the entire outer surface of the tooth, except for the apical 2 - 3 mm. with a barrier compound such as wax. Unfortunately, this still allows the dye solution to penetrate the multiple accessory canals usually present in the apex and, if left long enough, the dye will eventually pervade the dentin through the unwaxed cementum. Undetected gaps in the apical cementum will allow the intrusion of a test agent. Such penetration (by way of

the cementum and dentin) possibly could be interpreted as a failure of the restorative material or technic.

A number of other authors have placed their testing agent within the tooth.<sup>1,4,42,82,91</sup> Here, we are assured of assaying the marginal integrity of the material or technic employed in its placement as the tooth acts as its own dye reservoir and naturally confines penetration to any interface between tooth and seal it may reach. Preliminary studies have revealed that in the case of extracted human teeth methylene blue will penetrate any patent opening, diffuse through cementum in time, through minute abrasions in the cementum, and areas of developmental grooves which are especially vulnerable in dog teeth. Once the dye reaches the dentin tubules it passes unimpeded to the root canal making the perception of its derivation dubious.

With in vivo studies temperature is a built-in control. While trying to simulate the dental environment, in vitro studies are subject to temperature fluctuations and dehydration problems which, in spite of conscientious efforts, are difficult to compensate for. The temperature within the dog's root is constant by comparison. A dog is not normally fed hot meals so the teeth are not subject to as wide a range of temperature changes while feeding. They do, however, drink cool water which may provide some temperature variation.

In addition, occlusal forces are experienced in the dog comparable in many respects to those occurring in humans.

A review of the significant studies of tooth-restoration margins reveals that: 1) results depend on the operator who places the restorations; 2) results vary according to the material being employed; 3) results rely on the depth and range of the particular dye or isotope selected; 4) results will differ when performed in vivo and in vitro; 5) results are affected if a restoration is tested immediately after placement or following the lapse of a period of time; 6) conflicting results are reported by separate authors evaluating the same materials; and 7) testing solutions invariably penetrate all restorations.

Isotopes have been shown to penetrate further than dye solutions. This is possible because the autoradiogram is able to demonstrate an unseen isotope whereas a dye must be present in sufficient amounts to be visible. This does not rule out the rationale of dyes in margin studies. In many respects dyes are considered superior to radioactive ions, i.e., molecular size, differences in chemical structure, chemical inertness, and their propensity to combine histochemically with a wide assortment of healthy or pathological tissues and organs.<sup>48,49</sup>

It may be safely assumed that many of today's philosophic disagreements over materials and techniques are due in part to the diversity of experimental designs and results in penetration studies. Although the argument for unfettered freedom in this regard is sound, it is also accurate to assert that some standardization of procedure is in order. The following might be considered:

1. The status of the teeth to be treated. Briefly, this would cover extracted, pulpless, and vital tooth data from experimental animals, and man, in both young and mature dentitions.
2. The diffusion ability of the test solutions in experimental animals and man, in both young and mature dentitions.
3. The isotopic or dye solution, its pH and temperature during the experiment as well as molecular size.
4. Competent knowledge of the chemical reactivity of the testing media with the substances to be evaluated.
5. Selection of a controlled material and root canal filling technic.
6. Waiting a specified length of time to allow the materials to fully set.

In today's endodontic literature there is a dearth of knowledge concerning the physical characteristics and predictable properties of gutta percha and silver points under different environmental and working conditions. This is but one of the items which create gaps in our present knowledge.

Current dental articles are replete with "good, better, or best" being stressed when comparisons are made of marginal seals afforded by the clinical methods of individual operators. The assumption by the reader is that the lower rated materials in any given study is not worthy to be used in his practice. The clinician may fail to appreciate that all materials and penetrants have not been tested under the same conditions, have not been placed under standardized routines, that different investigators have varying technical abilities, that the skill and familiarity of the research analyst varies with each study and/or material, and certainly, that the clinician's own capacity to duplicate a study's results is purely speculative. If such testing methods were infallible, we'd have abandoned gold foil, amalgam and certainly root canal therapy long ago.

Restorations knowingly placed for a research project conceivably may be more carefully done than those routinely done in everyday dental practice. It is equally unfair to condemn a material or technic when it is not manipulated by

someone familiar with its working qualities. For this reason, warm gutta percha and diffusion technics used to seal root canals were purposely excluded from this thesis. Without knowledge or skill in the use of the intricacies of their management, they might have been unjustly surveyed.

Investigations of the margins produced by various root canal fillings will be informative, but without standardization they must be considered as being valid only for those operators placing the root canal fillings. Rather than adopting a technic which a particular article has deemed "best" under certain circumscribed conditions, we might better test our own ability utilizing the various technics. It is a simple matter to prepare and endodontically seal root canals with recommended or personal innovative modes, then seal the occlusal access opening, coat the tooth with wax and immerse the tooth in a vegetable dye or ink for 24-48 hours in an office incubator. The teeth can be longitudinally sectioned and examined. This would serve as a "spot-check" of our own mastery of a particular technic.

Endodontic root canal filling technics involving gutta percha with Kloroperka or chloropercha solutions were included for study since they are frequently condemned by penetration studies. Their condemnation has compelled many endodontists to use them almost furtively, as if they were

clandestine operations. Both materials are often resorted to by the endodontic community in particular "problem" situations. Their use may be indicated in: 1) narrow, ledged, or perforated canals, or canals obstructed by calcification, or broken instruments. They may also find use where an accessory canal or internal resorption is suspected. Some operators have found them helpful when adapting a master gutta percha cone or when obliterating ribbon - shaped canals, often encountered in mandibular anterior teeth. A tapered preparation, unviolated apical constriction and well dehydrated wall are emphasized as being fundamental to their effectiveness.

Although the Kloroperka technic had the highest mean linear penetration (4.14 mm.), its median penetration value was less than that for gutta percha, laterally condensed with thick or thin mixes of sealer (see Table 10).

The most obvious observation with the chloropercha technic is that when the methylene blue was placed on the same day as the filling procedure, it was able to penetrate less than half as far as when the dye was placed one week after sealing the root canal (1.49 mm. vs. 3.63 mm.). This finding is in agreement with that of Roydhouse and others.<sup>112,113,128,129</sup> Therefore, the results of dog # 298 lacked clinical reality and were omitted from the academic ranking in Table 10 of the modern methods of filling root

canals. Of the basic materials used to fill canals as measured by the standards of this study, chloropercha ranked second following the silver cone method.

The reason for the vivid increase in dye penetration in the root canals filled with chloropercha and allowed to age in situ, is openly speculative. Assuming that evaporation of the excess chloroform takes place, we might speculate that:

a) Reopening the tooth to place the dye allowed the evaporation to take place, or if these teeth had remained coronally sealed after root canal filling would their initial root canal seal be retained? b) How is evaporation accomplished in a completely sealed tooth, if it is? c) What is the coefficient of contraction or expansion of chloropercha upon setting or thermal changes?

Few authors have compared results of root canal fillings tested immediately upon filling versus those tested after remaining in situ for a period of time. Most studies were content to examine the comparative sealing potential of freshly condensed materials. In experiments where the investigator allowed the endodontic material to mature, increased penetration was generally observed. However, one study reported a decrease in penetration for a dye inserted after pausing for 48 hours.<sup>4</sup> The same authors also observed greater diffusion of their dye in zinc oxide-eugenol



cement than in Kloroperka. These are unique findings in the articles dealing with endodontics however.

Both the Kloroperka and chlorpercha fillings appeared to form a smooth, homogenous mass. Clinically, they appeared to adapt very well to the root canal's internal discrepancies. Its value in narrow, curved or unnegotiable canals where contraction is negligible may therefore be justified. Claims of these root canal fillings being good adherents to the dentinal walls are probably overstated as both poorly adhered. Of the two, Kloroperka root canal fillings seemed to cling to dentin somewhat better than chloropercha. Its mode of attachment was characteristically seen in patches. In extracted human teeth, the same observation was made during the preliminary tests of this project.

The intensity of the dentin stain penetrating beneath Kloroperka was judged as "heavy" while that beneath the chloropercha method was a bit less and deemed "moderately heavy".

For root canals filled solely with thick or thin mixes of Proco-Sol cement, the character of dentin staining is fairly consistent. The dentin stains "light" to "moderately heavy" at the superior reaches of the sealer. This intensity fades abruptly as one follows the stain apically. This contrasts with the pattern of fading produced

by the dye with Kloroperka and chloropercha restorations which usually fade gradually making the terminal penetration difficult to read.

When the root canals were filled with thick or thin Proco-Sol cement, they appeared to be clinically well-filled with the sealer up to the level of the orifice of the root canal. Rather remarkable differences in penetration occurred in those root canals filled with a hand-held Lentulo (1.77 mm. and 1.79 mm.) contrasted with the four controls in dogs # 331 and # 326 (3.83 mm.). The latter cement root canal fillings were placed by the commonly used means of turning a reamer or file counterclockwise in the root canal. In all these cases, the roots appeared to be satisfactorily and fully filled by the cement. Of interest here is that the four canals filled with the engine driven Lentulo experienced the least dye penetration of all the root canals in this study (1.25 mm.). They also were seen to be more compactly filled and embodied fewer voids. Where a canal is prepared large enough to accomodate it, the engine-operated Lentulo would appear to offer an excellent means of introducing root canal sealer into a tooth.

The penetrations of laterally condensed gutta percha employing thick or thin mixtures of root canal cement are close. There is a 0.30 mm. less linear penetration visible

when a thin mix was employed with gutta percha.

Upon re-examination of all our root canal fillings another item repeatedly entered the picture. Closer inspection of the preserved root canal fillings revealed that where the silver point filling method was used, 4 of the eight silver cones had distinct traces of dye well down their surfaces. Of these 4 restorations the average linear penetration was 3.43 mm. The mean penetration of the remaining 4 silver-filled canals was only 1.15 mm. In dog # 322 where a routine lateral condensation root canal filling technic was employed with gutta percha, strikingly similar results were observed. Here, 5 of the 6 bulk masses of retrieved gutta percha fillings had deposits of methylene blue on the surface of the gutta percha core. Again, gross penetration was also commonly seen in the dentin walls of these five canals. The average penetration of dye was 4.16 mm. compared to 1.2 mm. dye penetration in the lone root canal whose core of gutta percha was free of visible dye stain on the surface.

Coupled with the earlier observation that the dye appears to be able to penetrate only the surface 1.5 mm. of Proco-Sol cement, "penetration" may be a dual phenomenon where a bulk material or core is cemented into a root canal with a root canal sealer. The dye would appear to be able to "penetrate" through the silver/Proco-Sol and gutta percha/

Proco-Sol sealer interfaces. The deepest dye pattern on the dentin walls may therefore be a result of the dye's ability to permeate outward through narrow, adjacent thicknesses of the sealer (as would exist between the dentin wall and the well-condensed gutta percha). Here the dye's primary route of access was between the silver or gutta percha cone/Proco-Sol sealer interface. The lateral diffusion of the dye through the root canal sealer and onto the root canal wall may be termed "secondary penetration" in such cases.

An enigma was created when an attempt was made to equate or relate the intensity of the dentin's stain with the depth of linear penetration. When chloropercha and Kloroperka techniques were examined, "heavy" dye penetration was directly associated with a greater linear dye penetration. In general, this was not the case when a Proco-Sol sealer was involved in the root canal filling. With the lateral condensation technic the dye penetration was "light" but extended further toward the apex. Thus, when Proco-Sol sealer was a part of a root canal filling material, the intensity of methylene blue penetration was not directly proportional to the extent of linear penetration. No attempt was made to decode these notations, however, it is possibly related to the "secondary penetration" seen when Proco-Sol sealer is used with gutta percha or silver cones.

It is within the realm of possibility that dye was able to penetrate between the bulk materials and sealer if a less than adequate volume of sealer were used to coat the walls initially. This supposition would be more plausible if but a single cone of gutta percha were used. With multiple cones of gutta percha being laterally condensed, this supposition seems remote.

The controls where a single gutta percha or silver cone were adapted but seated without root canal sealer, were "very heavily" penetrated by the dye. Any attempt to restore a canal with a single cone, inspite of its sincere adaptation, would likely lead to failure.

Curiously enough, the most consistent dye-repelling root canal filling was Proco-Sol cement when used by itself. To avoid misinterpretation of this finding, we are reminded of the endodontic sealer studies of Curson and Kirk as well as those of Weiner and Schilder.<sup>35,138</sup> All, independently found that in time, the zinc oxide-eugenol based sealers deteriorate or volumetrically contract. Their solitary use as a complete sealant could also be expected to fail eventually.

It seems quite reasonable to believe that where root canal fillings are concerend, we are dealing with two interfaces: 1) the sealer/dentin interface and 2) the sealer/bulk filling material (core) interface. Non-union between

either one will admit apical tissue fluid into the canal system. Indigenous to root canal core materials and sealers as we know them today, there are known, inherent discrepancies in long term volume stability which complicate the problem.<sup>35,85,106,126,138</sup> An interpretation of those studies would imply that, upon setting, the Proco-Sol sealer contracts three-dimensionally (into itself). The interface becomes vulnerable as each root canal filling material (core and sealer) responds to:

1. The forces involved in the root canal filling technique.
2. Their innate properties (separate and/or in combination).
3. The influence imparted by the surrounding hard and soft tissue environment.

Future research would undoubtedly be rewarding if we knew more of the particulars of interface adhesion from studies of root canal filling materials, other dental materials and dentinal surfaces; from the inherent physical characteristics of root canal sealers in general and gutta percha in particular; and alternative materials and methods to isolate the root canal system from the periodontal ligament.

## B. Endodontic Aspects of Dog Teeth

Very little information is at hand covering the clinical endodontic aspects of dog teeth. A number of observations were made based on the animals treated endodontically during the course of this project and its preliminary studies. These comments are intended to help fill the broad gaps in the chasm of available clinical data, and hopefully, will assist the research of others in the future. The remarks are not intended to be a comprehensive treatise on canine endodontics but will be restricted to the maxillary and mandibular premolar therapy.

### General Observations

It goes without saying that a preliminary study of a dog skull will be helpful to familiarize the investigator with its general anatomic features and dental anatomy. At this time he may practice applying the rubber dam and trying various rubber dam clamps. This will reduce operating room time and preclude the possibility of not having suitable rubber dam clamps on hand. Practicing on extracted dog teeth prior to the main project will reap immeasurable benefits. These teeth may be obtained from schools of veterinary medicine, local veterinarians and from research facilities; i.e., hospitals, medical school, dental schools, etc.

During the preparatory dental exam when selecting a dog for endodontic research the occlusal surfaces should be inspected for wear patterns, chipped enamel, etc. Severely worn coronal surfaces are often associated with root canals which are comparatively small in size. At least four roots were deleted from this study due to inter-treatment tooth fractures. Tooth fractures due to food mastication was ruled out as a possible etiology in view of the fact that all of the test animals were fed soft diets during the evaluation period.

It may be that dogs confined to cages tend to gnaw to relieve their frustration or some may gnaw as extensions of a natural habit. Clues of a gnawing habit may be gained by examining the dentition and also the exposed bars of the cage for tooth marks. The act of gnawing contributes to the build-up of secondary dentin along the canal wall. This new dentin is very dense and was found to be intrinsically very hard making root canal preparation more difficult. This habitual grinding may also increase the difficulty of extracting these teeth and possibly contribute to ankylosis. Clinically, ankylosis was diagnosed as the cause of a minimum of three root fractures during the extraction process. In one case the entire root appeared to be rigidly attached to bone.



Inclusion of roentgenographs for instrumentation and case documentation is valuable. A portable type x-ray machine, manufactured and equipped specifically for dentistry is advisable. A 15 Ma portable hospital unit, however, may be adapted for dental use by adding a cone over the collimator. A few minutes taken before starting the operation will be well-spent on trial x-rays, varying the milliamperage and voltage settings and exposure times. This is especially advisable when different machines are provided. From one x-ray unit satisfactory roentgenographs were obtained with 90 Kvp at 10 Ma for 0.5 seconds. It is interesting to note that each article concerned with canine dentistry suggests different x-ray settings.<sup>107,111,134</sup>

One reason for favoring mandibular premolars in endodontic research is the inherent difficulty of taking maxillary roentgenographic views. The dog's palate is flat, therefore maxillary premolar and molar views must be taken from exaggerated angulations resulting in distorted images. This also applies to films of the mandibular cuspids and first and second premolars where the contour of the mandible's surface prevents routine film placement. An osseous shelf projects lingually from the inferior surface interfering with parallel film placement in these mandibular areas. Acceptable roentgenographic views of the maxillary and

mandibular incisors plus those of the mandibular premolar and molar areas may be obtained by following the usual dental x-ray methods. In these areas little anatomical interference is encountered.

Because of the difficulty in film placement, the packets are hand-held in hemostats. The body may be protected from direct radiation exposure by wearing protective aprons and gloves. For this technic the manufactured lead-lined mitten provides awkward hand control of the hemostat. The hand may be shielded from the direct rays by draping a protective cover over it. Another means would be to sew two or three strips of  $\frac{1}{2}$  inch flat, elastic ribbon on one side of the mitten which will stabilize it to the back of the hand leaving the fingers unencumbered.

#### External Premolar Anatomy

The dental formula for the dog's permanent dentition is normally  $2(I\frac{3}{3}, C\frac{1}{1}, P\frac{4}{4}, M\frac{2}{3})$  for a total of 42 teeth. However, some variation is seen according to the muzzle length of the animal. Brachycephalic (short-headed) dogs may have a decreased number of molar teeth. Carnassial (sectorial) is a term frequently applied to the maxillary fourth premolars and mandibular first molars.

The first premolars of either arch are usually single-rooted, peg-shaped teeth. They are small in stature,

averaging less than 14.0 mm. in length. The crown is rudimentary in appearance, often projecting less than 2.0 mm. into the dental arch. In older dogs the first premolars are frequently missing.

The second premolars were generally observed to have two roots. In all cases, this maxillary second premolar had two separate, divergent roots. In two of the ten animals, the roots of the mandibular second premolars were fused. Each segment, however, housed a separate pulp canal. In both instances the morphology was identical in the left and right quadrants, which also connotes bilateral symmetry. Our observation differs from that of Barker and Lockett who remarked that this tooth was "usually single rooted".<sup>6</sup>

The maxillary and mandibular third premolars, as well as the mandibular fourth premolars were found to continually possess two separate roots. From a dentist's point of view the maxillary fourth premolar appears to be misclassified (see Figure 17). It has large mesial and distal cusps, in addition to another cuspal projection on the mesiolingual portion of the crown. The root configuration of this carnassial tooth has three similarly corresponding roots. Clinically, the crown and root morphology of this upper fourth premolar suggests that it might be more appropriately included as a molar tooth.

In general, the distal roots of premolars are slightly shorter and more bulky in stature compared to the mesial roots. This contrast is especially noticeable in the mandibular premolars, however, is also true in the maxilla. As a general statement, it may be said that the root length increases with each premolar as one goes posteriorly (see Figure 18).

#### Internal Premolar Anatomy

Morphologically there appears to be little variability in the configuration of the premolar root canals from dog-to-dog and tooth-to-tooth. No individual root was seen to have more than a single canal. Unlike human teeth, no bizarre canal paths were discovered in canine premolars, for example, where a single canal bifurcates and later rejoins itself forming an island of dentin within the root canal. Although not encountered during the course of this project, the possible existence of diverse canal systems in canine premolars cannot be ruled out. The customary finding was a bulbous, sausage-shaped canal. Where the crown was chipped, heavily abraded and in older dogs, the lumen of the canals often appeared to be narrowed. In these cases the predentin or dentin was remarkably resistant to filing.

Prior to exiting, the apical pulp stump arborizes through multiple tiny foramina. As many as eight to ten

were counted in the horizontal ground sections. As few as two were noted, but less than four foramina was the exception rather than the rule. Unless one of the foramina was incidentally threaded, intentional perforation of the apex was arduous with hand filing. Barker and Lockett suggest the utilization of an engine-powered reamer for this purpose.<sup>6</sup>

### Extractions

Compared to man, the extraction of dog premolars presents an onerous task when the desire is to recover them intact. However, removal of teeth from younger dogs or those afflicted with periodontal disease does seem to require less physical effort. The extraction problem is partially due to the fact that very little lateral movement is possible with surgical elevators. The premolar roots have a longitudinal developmental groove on the interradicular surface. This groove becomes more pronounced as one proceeds posteriorly in the mouth and is especially evident on the distal roots. This groove acts as a "keyway" with the enveloping alveolar bone making a very stable niche for the tooth. This "keyway" also acts as a deterrent to rotational luxating moves.

The non-yielding nature of the alveolar bone surrounding the roots may prove to be the crux of the luxation difficulties. This conveys several potential facets. The

attachment apparatus may be more tenacious than in man. In addition, the histochemical composition of this bone may be more dense. The mandibular alveolar bone appears to contain only a modest amount of cancellous bone interposed between the buccal and lingual plates of cortical bone. Another observation was that the periapical roentgenograms generally revealed a broader, diffuse lamina dura circumscribing these roots. The lack of alveolar resiliency may be due in part or totally to these empirical notations.

In the surgical extraction process (see Figure 1) # 34 elevators are used to thoroughly loosen or expel the separated roots. The direction of this avulsion must be in the long axis of the teeth. Generally speaking, the maxillary premolars are easier to remove unscathed than those in the mandible. The reasons for this are not clear, however, it is conjectured that their shorter average root length (1 to 2 mm. less) and thinner buccal plate is implicated. Because of the existence of a flattened palate it can also be assumed that more cancellous-type bone surrounds the premolar roots of the upper arch.

Preliminary studies showed that, if intact, the dog's cementum will exclude methylene blue from the dentin for at least two days in vitro. It cannot be stated that this would be true in vivo. This observation may be of signifi-

cance in future in vivo endodontic studies because if the cementum is permeable to a dye, its penetration, although through the dentin, could readily be interpreted as due to "marginal penetration". Early tests in preparation for this thesis also indicated that cementum covering the roots of extracted human teeth is more readily penetrated with methylene blue than the cementum of dog's in vitro. Another observation from those early tests was that when this dye diffused through the cementum, in dog teeth, the area of the developmental groove was more susceptible to its entry. The dye's invasion into the dentin surrounding the groove usually assumed a wedge-shaped pattern (see Figure 19).

#### Longitudinal Sectioning

Sectioning the root in half vertically provides a superior means of examining the internal aspect of a root canal and/or its contents. It is also an excellent means of extracting the pulp for soft tissue studies. When this type of root division was adopted, longitudinal paths were cut as near to 180° circumferentially to one another as possible. The depth of both channels was cautiously prepared in order to penetrate close, to but not into, the root canal. It was discovered that when the long-axis grooves were not situated opposite to one another or close enough to the root canal, the blades of the side-cutting Rongeur (used to effect the

root's fracture) were directed obliquely through the root, producing an unfavorable split alongside the canal.

Occasionally this resulted in three root fragments, the third piece being the tubular frame of the canal. If the teeth originated from mature dogs, it could be theorized that cleavage occurred at the interface between the primary and secondary dentin. However, this could be averted by cutting two "starter" notches at the 12 and 6 o'clock positions occlusally and at the apex then simply uniting the two cuts on each side.



## CHAPTER VI

### SUMMARY

The purpose of this in vivo project was twofold:

1) to explore the possibility of utilizing intravenous injections of methylene blue for evaluating endodontic procedures; 2) to evaluate the relative penetrability of commonly used root canal filling materials and techniques in vivo with 1% methylene blue placed intradentally.

The experimental design involved two separate and distinct phases: PHASE I - Selected teeth were treated endodontically. A 1% methylene blue injection U.S.P. was given intravenously into four dogs. Several controlled injection techniques were attempted in an effort to attract the disclosing solution to the alveoli of the teeth. This included inducing mild periapical inflammation, multiple injection cycles, varying the concentration of the dye, bypassing the kidneys, and placing the dye near the apices in intrabony reservoirs. Histologic samples of pulp and gingival tissues were examined for their gross content of dye following the various injection techniques. The teeth were scrutinized externally and internally for the presence of the dye. Insufficient quantities of the 1% methylene blue

reached the dental apparatus to validly judge endodontic aspects of dental treatment.

In PHASE II a total of 88 root canals in six dogs were endodontically treated to observe the response of several materials and technics manipulated in the teeth of a living model. These methods and treatments were utilized in the same manner as they would be clinically. The compositions were allowed to mature in situ for one week. The 1% methylene blue injection, U.S.P. was then sealed in an intradental crypt atop the endodontic restorations for 48 hours. In one dog the dye was deposited on the same day as the filling procedure. The teeth were surgically extracted in order to recover them intact. The roots were then sectioned vertically in half. The root canal preparation, basic filling material, root canal sealer and character of the dye penetration, could be visually inspected throughout the breadth and length of the root canal.

In addition to the biologic model having root canals similar to man's and following procedures as used in the office, the experiments were controlled with the following parameters: The specific root canal filling material and filling technics were performed by persons clinically familiar with their nuances; the same type of root canal filling was placed in a single animal; each type of root

canal filling was inserted by one individual on a given day; the dye was systematically deposited; and the entire root canal filling and its sealing ability could be circumspected by vertically sectioning through each root canal.

From the data of this study the following observations may be made:

- 1) Intravenous injections of methylene blue as attempted in this investigation is an inadequate means of evaluating endodontic procedures.
- 2) Untoward reactions may occur in animals even when "non-toxic" dyes are employed intravenously. These responses have occurred with the use of refined or "medicinal grades" of dye such as 1% methylene blue injection, U.S.P., in dogs.
- 3) These results represent a "potential" sealing ability of the root canal filling materials. Each person cannot expect to get precisely the same results because of varying innate personal abilities. The manner of employing each root canal filling material will vary from one individual to the next. Clinical results reflect an individual's ability and conscience as well as the potential quality of a root canal filling material and technic. Many discrepancies and poor performance attributed to a material in penetration studies are just as likely to be due to the operator as the root canal filling materials.

- 4) Variable results may be realized from the same root canal filling material and technic even when manipulated by the same operator. The quality of one individual's root canal fillings will vary throughout a given day.
- 5) The consistency has little to do with the seal provided by Proco-Sol radiopaque silver cement. The finding that relatively good sealing results are obtained even when root canals are poorly filled, voids exist, etc., suggests that this sealer may possess a partial chemical inhibition toward methylene blue. This study has seen that the dye will penetrate nearly 1.5 mm. of the sealer's surface. Another clue that a chemical barrier may be operating is the observation that the vertical stain pattern fades abruptly rather than gradually when the dentin surface is protected with Proco-Sol cement.
- 6) In endodontics we are often dealing with two interfaces:
  - a. Between the root canal sealer and tooth surface.
  - b. Between the root canal sealer and bulk filling material (gutta percha or silver cones).

A clinical case may fail due to penetration through either route. These interfaces become penetrable as a result of: manipulation, differences in volumetric stability and surface characteristics peculiar to each substance.

- 7) To realistically evaluate any root canal filling material or technic in vivo or in vitro, ample time must be given to allow the root canal filling materials to thoroughly set before exposing it to a test design.
- 8) A solution of 1% methylene blue injection, U.S.P., is capable of penetrating any root canal filling materials or techniques as utilized during this investigation.
- 9) None of the root canal filling techniques as evaluated by this investigation may be justly endorsed nor renounced as good, better or best, worst, etc. Each root canal filling technique has its own rate of success according to the capability of the clinician. Judgement of the sealability of root canal filling materials and techniques cannot be based on numerical values alone.
- 10) As yet, dentistry has not seen the "ideal" endodontic root canal filling material or technique. If it has, man and nature have expunged its perfection.

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## CHAPTER VIII

### APPENDIX

A. Tables: -----

B. Figures: -----

TABLE 1

I.D. # 166 - SEGMENTED GUTTA PERCHA WITH KLOROPERKA N-O

<u>Tooth</u>	<u>Restoration</u>	<u>Prep. Length (mm.)</u>	<u>Linear Pentr. (mm.)</u>	<u>% Pentr.</u>
Right Mand.				
2nd M	Prep. only (Control)	7.3	7.3	100.00
D	Prep. only (Control)	6.5	6.5	100.00
3rd M	G.P. + Kloro.	7.6	3.6	47.37
D	"	6.8	3.8	55.88
4th M	"	---	---	---
D	"	7.4	2.6	35.14
Left Mand.				
2nd M	Prep. only (Control)	---	---	---
D	Prep. only (Control)	6.5	6.5	100.00
3rd M	G.P. + Kloro.	6.8	3.9	57.35
D	"	7.3	5.4	73.97
4th M	"	6.8	3.0	44.12
D	"	8.5	6.5	78.82
Total # Rests = 8				
Total # Controls = 3				
Total Length - Rests.		59.9	29.0	48.41
" " - Controls		20.3	20.3	100.00
Ave. " - Rests.		8.56	4.14	28.36
" " - Controls		6.77	6.77	100.00
Total % Penetr. - Rests.				392.65
" " " - Controls				300.00
Ave. " " - Rests.				56.09
" " " - Controls				100.00

TABLE 2

I.D. # 166 - PROCO-SOL (THICK)

<u>Tooth</u>	<u>Restoration</u>	<u>Prep. Length (mm.)</u>	<u>Linear Pentr. (mm.)</u>	<u>% Pentr.</u>
Right Max.				
2nd M	Proco-Sol (thick*)	6.6	.8	12.12
D	Proco-Sol (thick*)	5.7	2.5	43.86
3rd M	Proco-Sol (thick*)	6.6	1.0	15.15
D	Proco-Sol (thick*)	6.7	2.4	35.82
Left Max.				
2nd M	Proco-Sol (thick*)	---	---	---
D	Proco-Sol (thick*)	5.9	0.7	11.86
3rd M	Proco-Sol (thick*)	6.7	2.6	38.81
D	Proco-Sol (thick*)	5.6	2.4	42.86

Total # Rests. = 8

Total # Controls = 3

Total Length - Rests. 43.8

" " - Controls 20.30

Ave. " - Rests. 6.26

" " - Controls 6.77

Total % Penetr. - Rests.

" " " - Control

Ave. " " - Rests.

" " " - Controls

12.4 28.31

20.30 100.00

1.77 28.27

6.77 100.00

200.48

300.00

28.64

100.00

\* Sealer placed via hand-held Lentulo in this animal.

TABLE 3

I.D. # 298 - SEGMENTED GUTTA PERCHA WITH CHLOROPERCHA

<u>Tooth</u>	<u>Restoration</u>	<u>Prep. Length (mm.)</u>	<u>Linear Pentr. (mm.)</u>	<u>% Pentr.</u>
Right Mand.				
2nd M	Prep. only (control)	7.1	7.1	100.00
D	Prep. only (control)	7.6	7.6	100.00
3rd M	G.P. + Chloro.	6.6	0.7	10.61
D	"	6.3	0.9	14.29
4th M	"	7.4	0.6	8.11
D	"	8.2	3.1	37.80
Left Mand.				
2nd M	Prep. only (control)	6.7	6.7	100.00
D	Prep. only (control)	6.8	6.8	100.00
3rd M	G.P. + Chloro.	5.4	1.7	31.48
D	"	5.8	1.7	29.31
4th M	"	7.4	1.7	22.97
D	"	7.8	1.5	19.23
Total # Rests. = 8				
Total # Controls = 4				
Total Length - Rests.		54.90	11.90	21.68
" " - Controls		28.20	28.20	100.00
Ave. " - Rests.		6.86	1.49	21.68
" " - Controls		7.05	7.05	100.00
Total % Penetr. - Rests.				173.80
" " " - Controls				400.00
Ave. " " - Rests.				21.73
" " " - Controls				100.00

TABLE 4

I.D. # 301 - SEGMENTED GUTTA PERCHA WITH CHLOROPERCHA

<u>Tooth</u>	<u>Restoration</u>	<u>Prep. Length (mm.)</u>	<u>Linear Pentr. (mm.)</u>	<u>% Pentr.</u>
Right Mand.				
2nd M	Prep. only (control)	---	---	---
D	Prep. only (control)	---	---	---
3rd M	G.P. + Chloro.	7.3	3.8	52.05
D	"	6.0	2.4	40.00
4th M	"	8.1	4.8	59.26
D	"	8.7	7.5	86.21
Left Mand.				
2nd M	Prep. only (control)	8.5	8.5	100.00
D	Prep. only (control)	7.3	7.3	100.00
3rd M	G.P. + Chloro.	6.4	3.6	56.25
D	"	6.0	3.1	51.67
4th M	"	7.0	1.8	25.71
D	"	7.6	2.0	26.32
Total # Rests. = 8				
Total # Controls = 2				
Total Length - Rests.		57.1	29.0	50.78
" " - Controls		15.8	15.8	100.00
Ave. " - Rests.		7.14	3.63	50.84
" " - Controls		7.9	7.95	100.00
Total % Penetr. - Rests.				397.47
" " " - Controls				200.00
Ave. " " - Rests.				49.68
" " " - Controls				100.00

TABLE 5

I.D. # 301 - PROCO-SOL (THIN)

<u>Tooth</u>	<u>Restoration</u>	<u>Prep. Length (mm.)</u>	<u>Linear Penetr. (mm.)</u>	<u>% Penetr.</u>
Right Max.				
2nd M	Proco-Sol (thin*)	6.8	2.0	29.41
D	Proco-Sol (thin*)	6.1	3.4	55.74
3rd M	Proco-Sol (thin*)	7.5	1.7	22.67
D	Proco-Sol (thin*)	6.5	1.4	21.54
Left Max.				
2nd M	Proco-Sol (thin*)	6.5	1.0	15.38
D	Proco-Sol (thin*)	7.3	3.1	42.47
3rd M	Proco-Sol (thin*)	6.4	0.9	14.06
D	Proco-Sol (thin*)	6.5	0.8	12.31
Total # Rests. = 8				
Total # Control = 2				
Total Length - Rests.		53.60	14.3	26.68
" " - Controls		15.8	15.8	100.00
Ave. " - Rests.		6.7	1.79	26.72
" " - Controls		7.9	7.95	100.00
Total % Penetr. - Rests.				213.58
" " " - Controls				200.00
Ave. " " - Rests.				26.70
" " " - Controls				100.00

\* Sealer placed via hand-held Lentulo in this animal.

TABLE 6

I.D. # 322 - GUTTA PERCHA WITH PROCO-SOL (THIN) - (LAT. COND.)

<u>Tooth</u>	<u>Restoration</u>	<u>Prep. Length (mm.)</u>	<u>Linear Penetr. (mm.)</u>	<u>% Penetr.</u>
Right Mand.				
2nd M	Thin Proc. (Eng.*)	7.1	2.0	28.17
D	Thin Proc. (Eng.*)	5.9	1.1	18.64
3rd M	G.P. + Proc.	---	---	---
D	"	6.7	4.3	64.18
4th M	"	7.4	2.7	36.49
D	"	7.2	4.6	63.89
Left Mand.				
2nd M	Thin Proc. (Eng.*)	5.3	1.2	22.64
D	Thin Proc. (Eng.*)	5.7	0.7	12.28
3rd M	G.P. + Proc.	---	---	---
D	"	5.9	1.2	20.34
4th M	"	8.5	4.3	50.59
D	"	7.7	4.8	62.34

Total # Rests. = 6

Total # Controls = 4

Total Length - Rests. 43.40 21.9 50.46

" " - Controls 24.00 5.0 20.83

Ave. " - Rests. 7.23 3.65 50.48

" " - Controls 6.00 1.25 20.83

Total % Penetr. - Rests. 297.83

" " " - Controls 81.73

Ave. " " - Rests. 49.64

" " " - Controls 20.43

\* Sealer placed via dental handpiece-driven lentulo in this animal.



TABLE 7

I.D. # 326 - SILVER POINTS WITH PROCO-SOL (THICK)

<u>Tooth</u>	<u>Restoration</u>	<u>Prep. Length (mm.)</u>	<u>Linear Pentr. (mm.)</u>	<u>% Pentr.</u>
Right Mand.				
2nd M	Thick Proc. (control*)	6.5	5.0	76.92
D	Ag. Pt. only (control)	7.1	6.1	85.92
3rd M	Ag. Pt. + Proc.*	7.4	0.9	12.16
D	"	7.4	3.7	50.00
4th M	"	7.4	2.8	37.84
D	"	7.7	0.3	3.90
Left Mand.				
2nd M	Thick Proc. (control*)	6.9	3.3	47.83
D	Ag. Pt. only (control)	7.1	3.8	53.52
3rd M	Ag. Pt. + Proc.*	7.4	4.4	59.46
D	"	7.3	2.6	35.62
4th M	"	7.7	0.6	7.79
D	"	7.0	3.0	42.86

\* Sealer placed via hand-held root canal file in this animal.

Total # Rests. = 8				
Total # Controls = 2 + 2				
Total Length - Rests.	59.3	18.3	30.86	
" " - Proc. (thick)	13.4	8.3	61.94	
" " - Ag. Pt. (only)	14.2	9.9	69.72	
Ave. " - Rests.	7.41	2.29	30.87	
" " - Proc. (thick)	6.7	4.15	61.94	
" " - Ag. Pt. (only)	7.1	4.95	69.72	
Total % Penetr. - Rests.			249.63	
" " " - Proc. (thick)			124.75	
" " " - Ag. Pt. (only)			139.44	
Ave. % Penetr. - Rests.			31.20	
" " " - Proc.			62.38	
" " " - Ag. Pt. (only)			69.72	

TABLE 8

I.D. # 331 - GUTTA PERCHA WITH PROCO-SOL (THICK) - (LAT. COND.)

<u>Tooth</u>	<u>Restoration</u>	<u>Prep. Length (mm.)</u>	<u>Linear Pentr. (mm.)</u>	<u>% Pentr.</u>
Right Mand.				
2nd M	Thick Proc. (control*)	7.4	5.6	75.68
D	G.P. (control)	5.3	5.3	100.00
3rd M	G.P. + Proc. (thick*)	6.5	2.6	40.00
D	G.P. + Proc. (thick*)	6.5	5.6	86.15
4th M	G.P. + Proc. (thick*)	7.6	3.4	44.74
D	G.P. + Proc. (thick*)	8.1	4.5	55.56
Left Mand.				
2nd M	Thick Proc. (control*)	6.5	1.6	24.62
D	G.P. (control)	5.1	5.1	100.00
3rd M	G.P. + Proc. (thick*)	7.3	2.3	31.51
D	G.P. + Proc. (thick*)	7.0	2.8	40.00
4th M	G.P. + Proc. (thick*)	7.7	5.8	75.32
D	G.P. + Proc. (thick*)	8.2	4.6	56.10

\* Sealer placed via hand-held root canal file in this animal.

Total # Rests. = 8

Total # Controls = 2 + 2

Total Length - Rests.	58.9	31.6	53.65
" " - Proc. (thick)	13.9	7.2	51.79
" " - G.P. (only)	10.4	10.4	100.00
Ave. " - Rests.	7.36	3.95	53.67
" " - Proc. (thick)	6.95	3.6	52.79
" " - G.P. (only)	5.2	5.2	100.00

continued on next page

TABLE 8 (cont'd.)

Total	%	Penetr.	-	Rests.	429.38
"	"	"	-	Proc. (thick)	100.30
"	"	"	-	G.P. (only)	200.00
Ave.	"	"	-	Rests.	53.67
"	"	"	-	Proc. (thick)	50.15
"	"	"	-	G.P. (only)	100.00

TABLE 9

RANK ACCORDING TO LINEAR PENETRATION

	<u>Linear Penetr. (mm.)</u>	<u>Intensity of Penetr.</u>
1. Thin Proc. (#322)*	1.25 (4)	mod. heavy
2. Gt. Pch. + Chloro. (#298 - Immed. dye)	1.49 (8)	very light
3. Thick Proc. (#166)**	1.77 (7)	mod. heavy
4. Thin Proc. (#301)**	1.79 (8)	light
5. Ag. Pt. + Thick Proc. (#326)***	2.29 (8)	very light
6. Gt. Pch. + Chloro. (#301 aged rests.)	3.63 (8)	mod. heavy
7. Gt. Pch. + Thin Proc. (#322)*	3.65 (6)	mod. heavy
8. Thick Proc. (controls of #326 + 331)***	3.83 (4)	heavy
9. Gt. Pch. + Thick Proc. (#331)***	3.95 (8)	heavy
10. Gt. Pch. + Kloro. (#166)	4.14 (7)	heavy
11. (1) Ag. Pt. alone (#326)	4.95 (2)	very heavy
12. (1) Gt. Pch. cone alone	5.20 (2)	very heavy
13. Prep. - no fills (controls of #166, 298 & 301)	7.14 (7)	very heavy
	↑ (# rests.)	

\* Sealer placed via dental handpiece-driven Lentulo.

\*\* Sealer placed via hand-held Lentulo.

\*\*\* Sealer placed via hand-held root canal file.

Comparative Intensity Range:

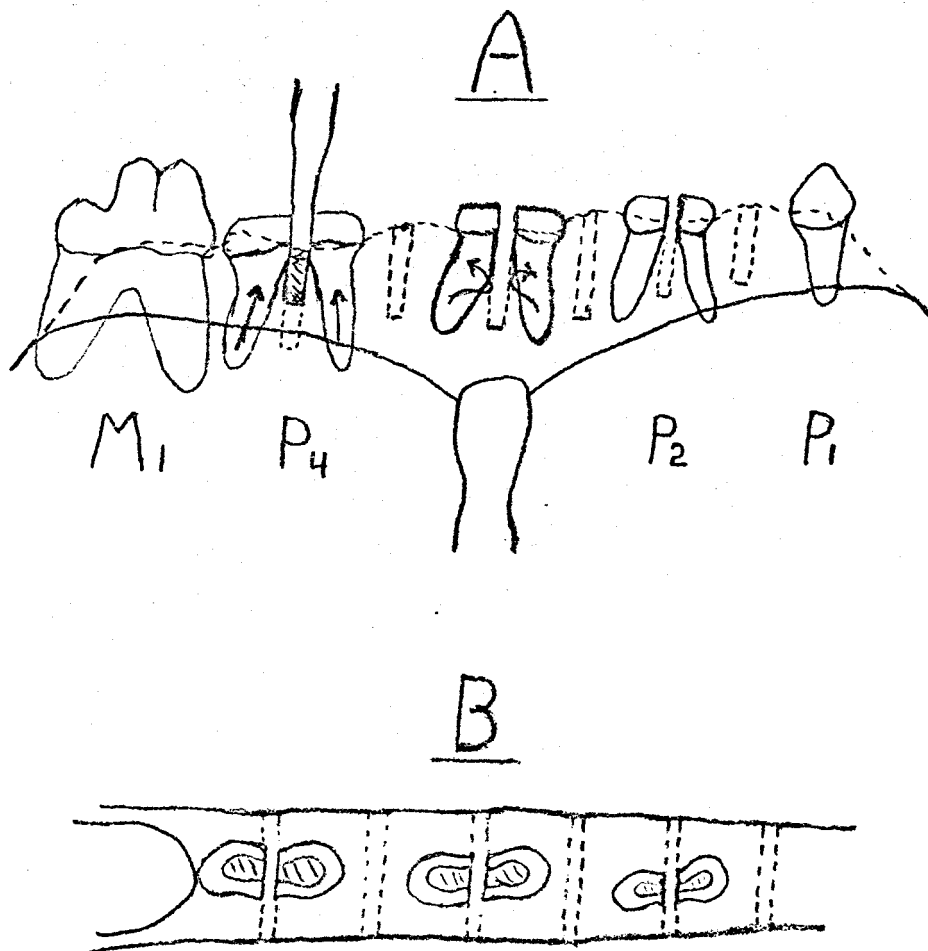
1. very light    2. light    3. mod. heavy    4. heavy    5. very heavy

TABLE 10

RANK -- BY RESTORATION TECHNIQUE

	<u># of Rests.</u>	<u>Range of Linear Penetr. (mm.)</u>	<u>Median Linear Penetr. (mm.)</u>	<u>Mean Linear Penetr.(mm.)</u>
1. Ag. Pt. + Proc. (#326)	8	0.3 - 4.4	2.7	2.29
2. Gt. Pch. + Chloro. (#301)	8	1.8 - 7.5	3.35	3.63
3. Gt. Pch. + Proc. (thin) (#322)	6	1.2 - 4.8	4.3	3.65
4. Gt. Pch. + Proc. (thick) (#331)	8	2.3 - 5.8	3.95	3.95
5. Gt. Pch. + Kloro. (#166)	7	2.6 - 6.7	3.8	4.14

FIGURE 1

SURGICAL EXTRACTION TECHNIQUE

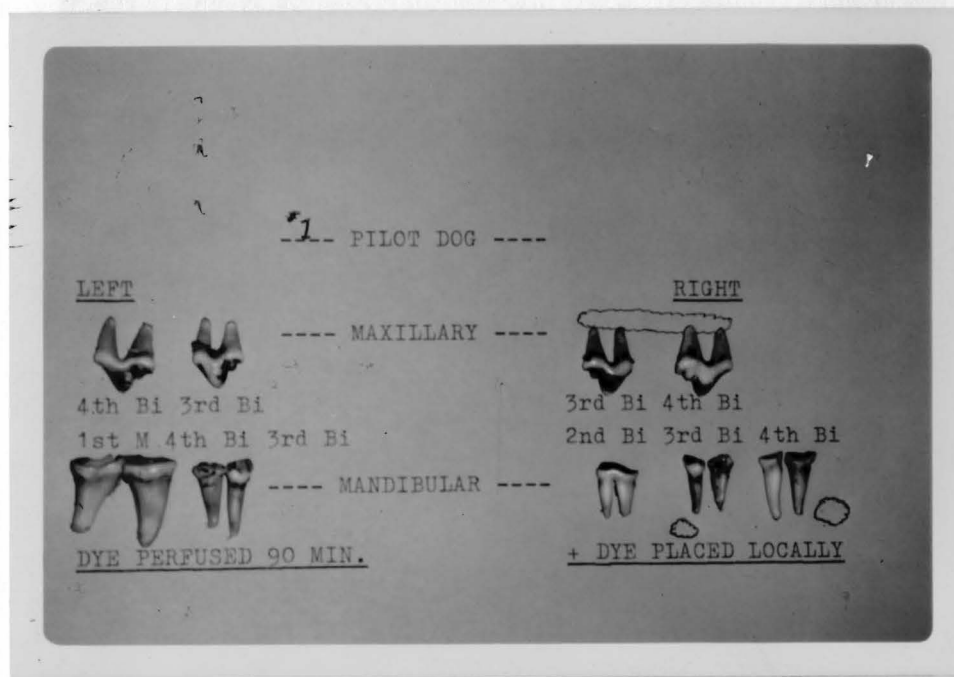
A. Facial View  
B. Occlusal View

FIGURE 2

X-RAY ADAPTED FOR DENTAL USE

FIGURE 3

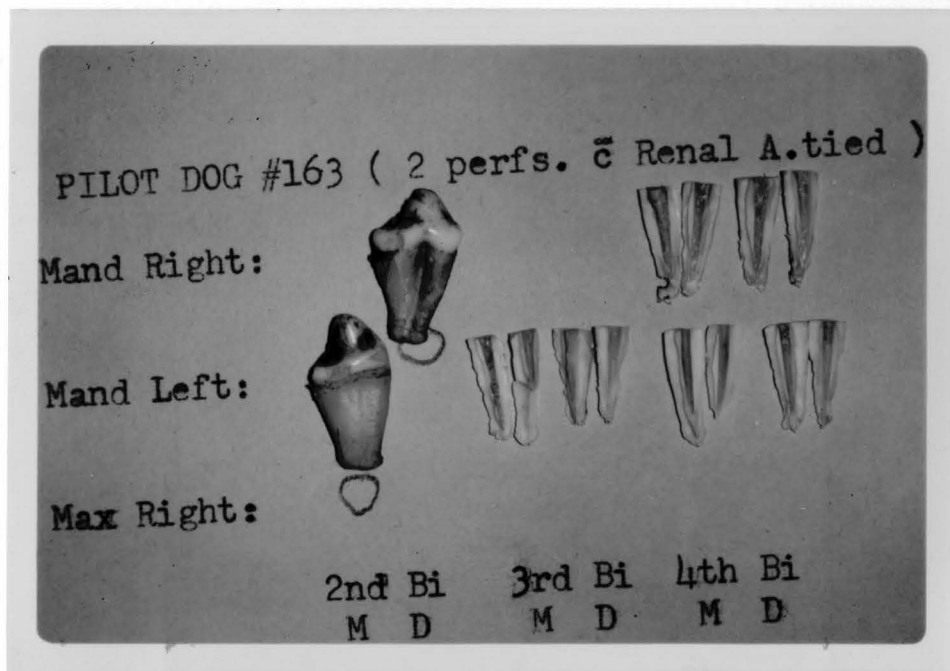
I.D. #713: PILOT DOG #1 - 90 MINUTE PERFUSION



- Note:
1. Dog died 2 hours after dye perfusion began.
  2. Max. left premolar extracted 30 minutes after death.
  3. "Clouds" indicate dye reservoirs created in bone.  
Dye placed in reservoirs for 24 hours.



I.D. #163: 2 DYE PERFUSIONS, KIDNEYS BYPASSED



- Note:
1. Canals filled via lateral condensation.
  2. "Clouds" indicate the approx. position of the dye reservoir to the apex.
  3. Degree of stain on root surface.

FIGURE 5

I.D. #163: DISTRIBUTION OF DYE IN ORAL TISSUES



- Note:
1. Author regrets poor color reproduction from slide.
  2. Coloration of marginal gingiva, inferior surface of tongue and tonsillar area.
  3. Flat palate in dog.

FIGURE 6

I.D. #163: ANGIONEUROTIC EDEMA

Note: 1. Allergic wheals arising from dermis on inner aspect of hind legs.

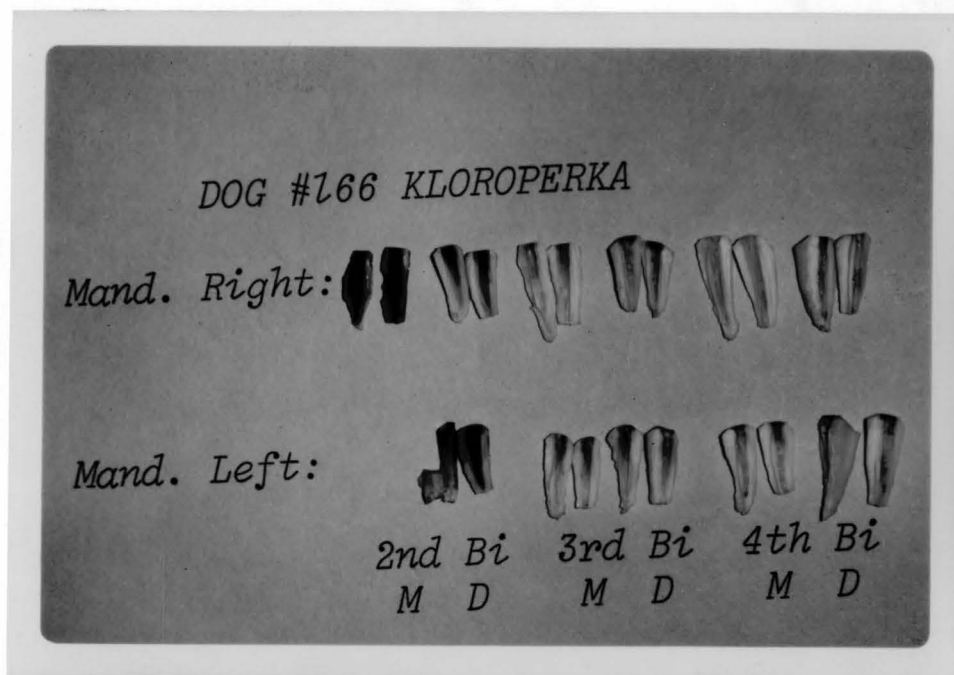
FIGURE 7

I.D. #163: PERIPHERAL VASCULAR COLLAPSE



- Note: 1. Intracath in External Jugular Vein.  
2. Collapse caused cessation of dye flow with 15cc.  
solution remaining in Buretrol.

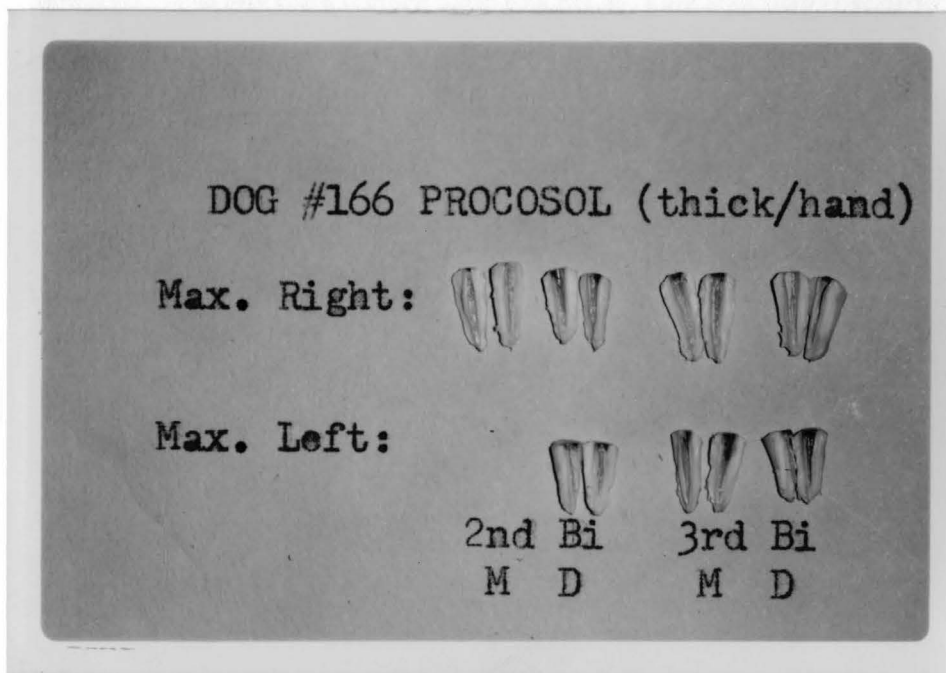
FIGURE 8

I.D. #166: PENETRATION OF KLOROPERKA ROOT CANAL FILLINGS

- Note:
1. Dye distribution in each half section.
  2. 2nd premolars prepared but not filled.
  3. Dye pattern in left 4th premolar (D)
  4. Right 4th premolar (M) deleted from study because of improper dye placement.

FIGURE 9

I.D. #166: PENETRATION OF PROCO-SOL (thick)

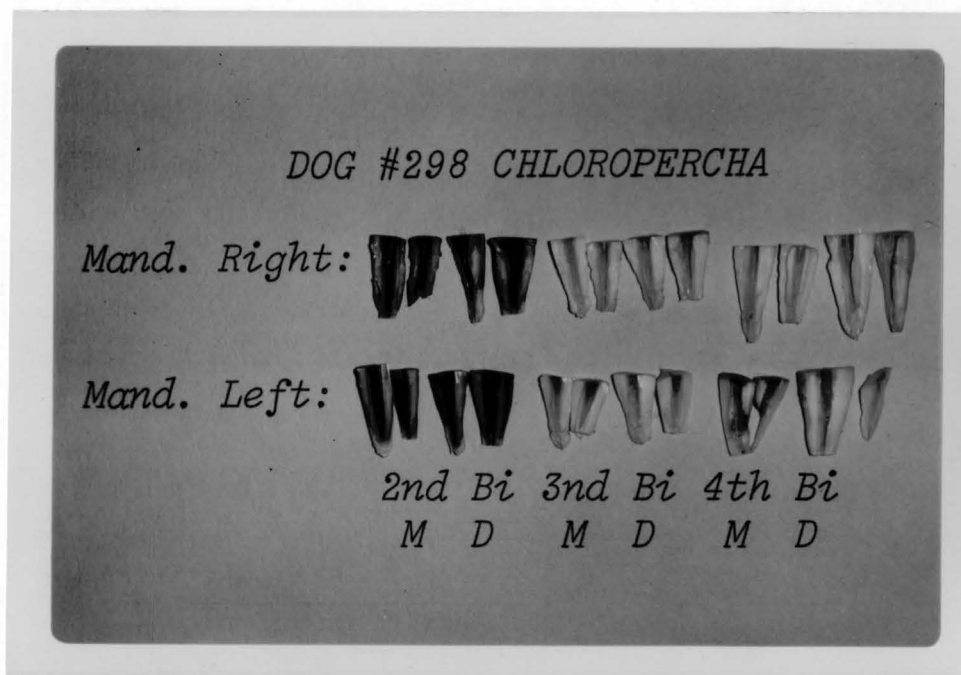


Note: 1. Extent of stain on root canal wall.  
2. Rapid fading of dye's intensity.



FIGURE 10

I.D. #298: PENETRABILITY OF CHLOROPERCHA ROOT CANAL FILLING

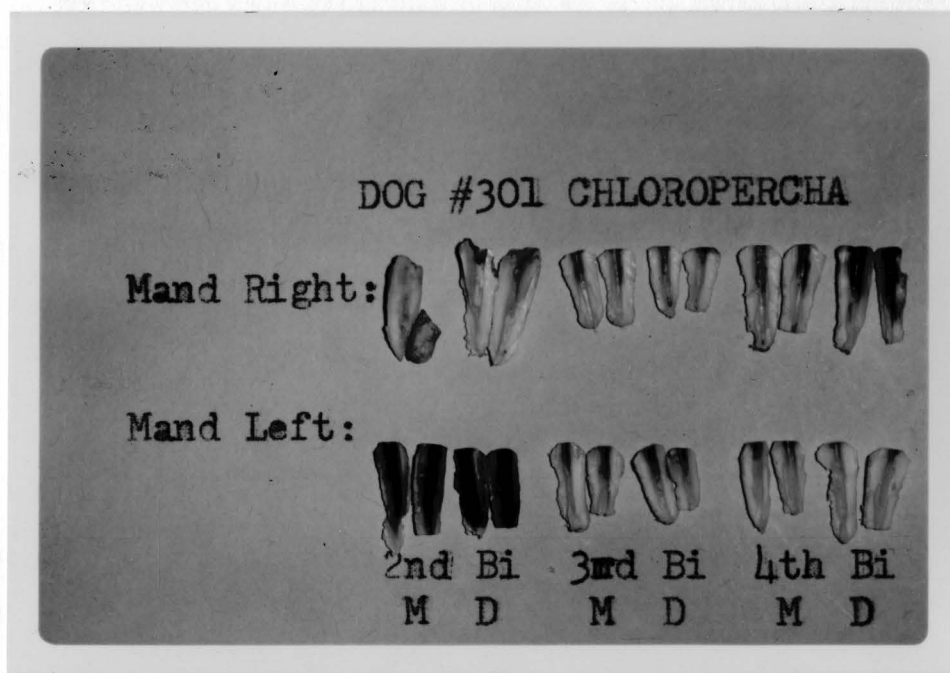


- Note: 1. Dye placed on the same day as the root canal filling.  
 2. Relative lack of dye's vertical (linear) penetration.  
 3. Dye intensely pervaded control teeth.  
 4. Apical cementum relatively free of dye.

1. Increased linear penetration compared to #298. Root canal filling here was allowed to "age" for 1 week.

FIGURE 11

I.D. "301: PENETRABILITY OF CHLOROPERCHA ROOT CANAL FILLING

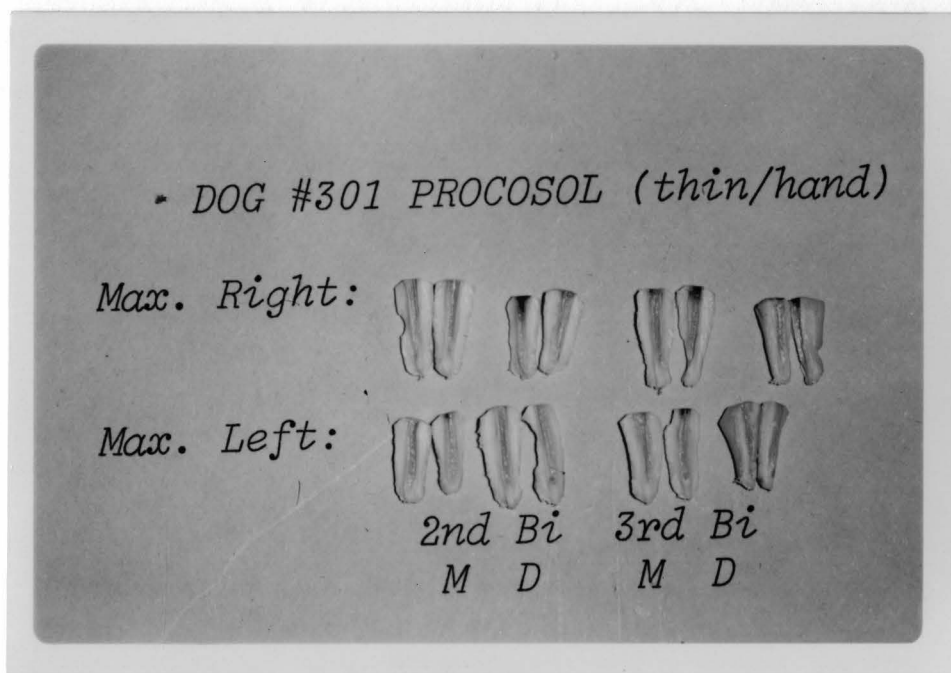


- Note:
1. Mand. right 2nd premolar fractured on cage bars and deleted from study.
  2. Identical dye staining in control tooth here as with #298.
  3. Increased linear penetration compared to #298. Root canal filling here was allowed to "age" for 1 week.



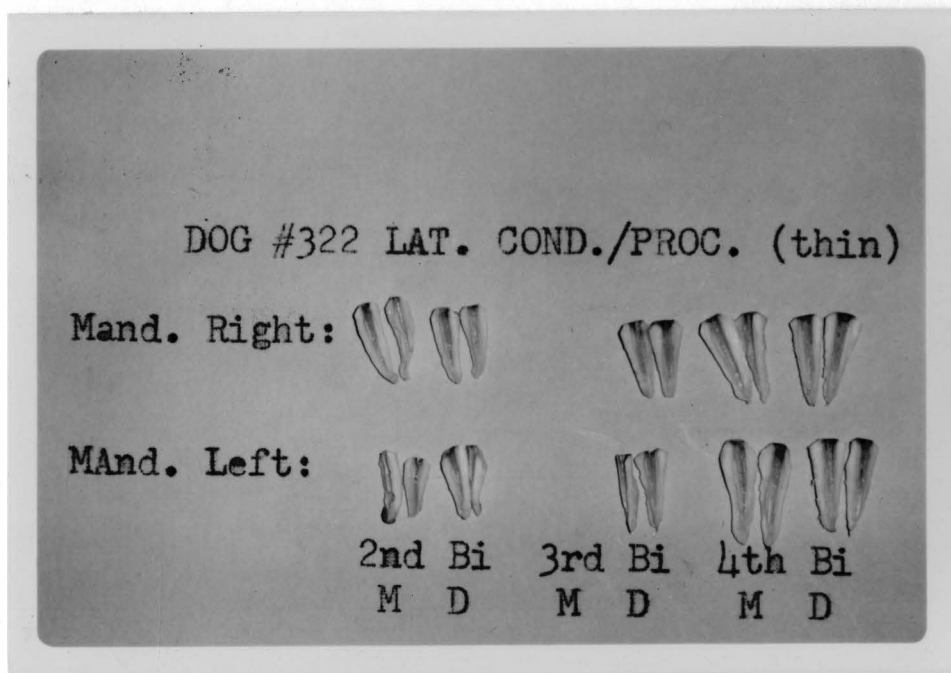
FIGURE 12

I.D. #301: PENETRABILITY OF PROCO-SOL (thin)



- Note: 1. "Heavy" initial penetration but rapidly fades as it extends apically.
2. Extensive but "light" penetration on root canal wall of the right 1st (B) premolar.
3. Collectively, the 4 roots of these 2nd premolars served as controls (sealer placed with hand-driven lentulo) and exhibited the least linear penetration in this study.
4. The left 3rd premolar (B) was the only filling (laterally condensed) in which the sealer was not evident on the gutta-percha's surface.

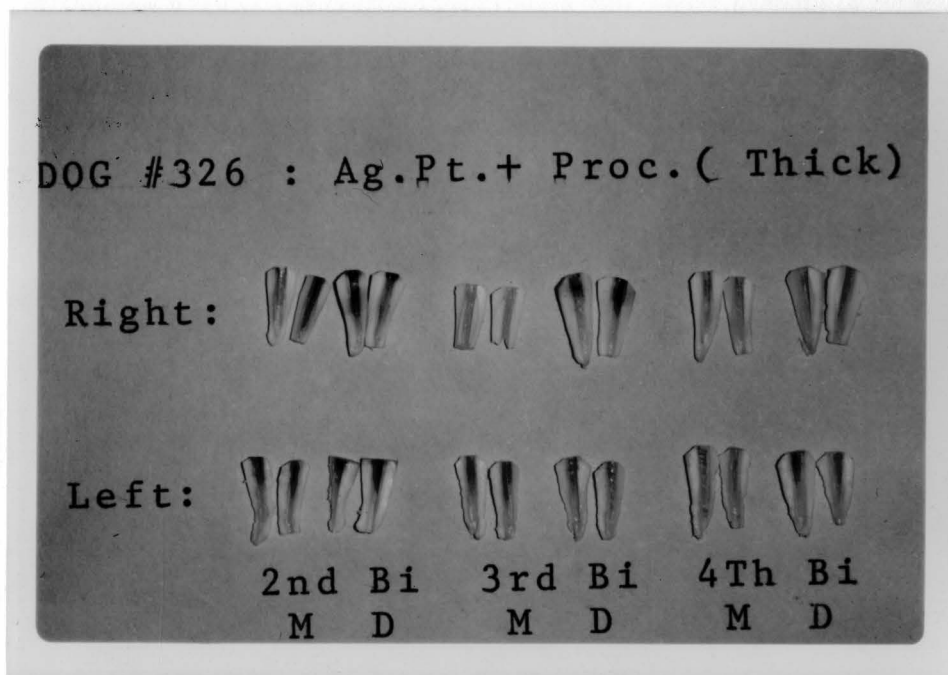
FIGURE 13

I.D. #322: PENETRABILITY OF LATERALLY CONDENSED GUTTA PERCHA

- Note:
1. Mesial roots of both 3rd premolars deleted from study due to their fracture by the dog on his cage bars. Interestingly, both roots fractured along their long axis.
  2. Extensive but "light" penetration on root canal wall of the right 3rd (D) premolar.
  3. Collectively, the 4 roots of these 2nd premolars served as controls (sealer placed with handpiece-driven Lentulo) and exhibited the least linear penetration in this study.
  4. The left 3rd premolar (D) was the only filling (laterally condensed) in which dye was not evident on the gutta percha's surface.

FIGURE 14

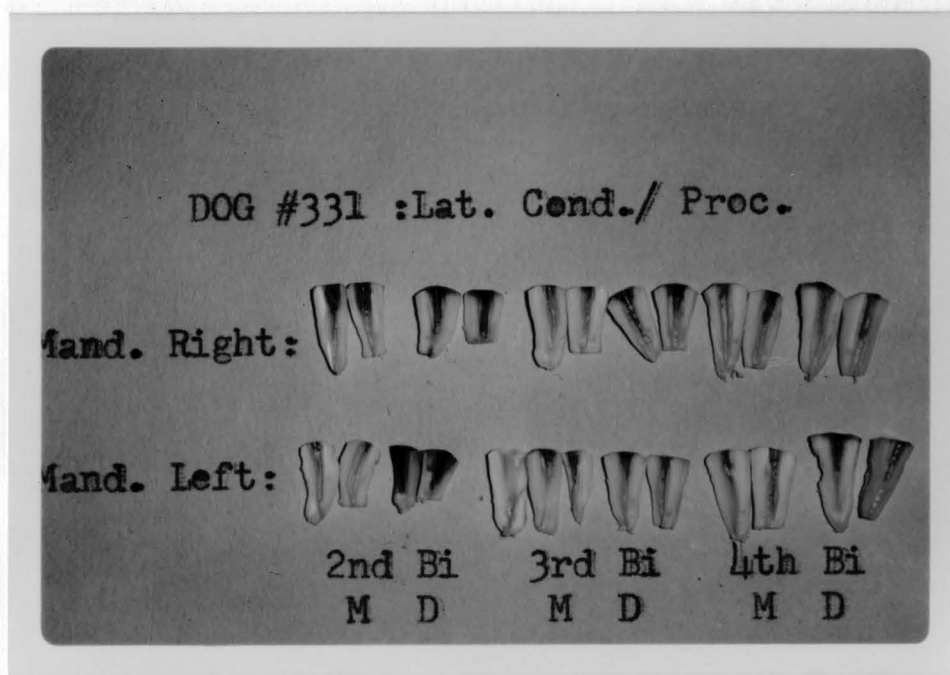
I.D. #326: PENETRABILITY OF SILVER POINT TECHNIC



- Note:
1. These root canal fillings exhibited the lowest mean linear penetration of the popular technics tested in this study.
  2. Dye stain was observed on the silver's surface with the right 3rd (M) and the left side's 3rd (M&D) and the 4th (D).
  3. The "lighter" intensity generally present for those listed in the above notations.

FIGURE 15

I.D. #331: PENETRABILITY OF LATERALLY CONDENSED GUTTA  
PERCHA (thick Proc.)



- Note: 1. Wide variations in the extent of linear penetration from one specimen to another.
2. The left and right 2nd (D) were filled with 1 adapted gutta percha cone, but without sealer.
3. The differences in character of the dye's diffusion pattern in the left and right 2nd (M) where both were filled through the use of hand-held root canal files.
4. "Test tube" fragmentation of left 3rd (M) mentioned in this thesis under "Endodontic Aspects of Dog Teeth".

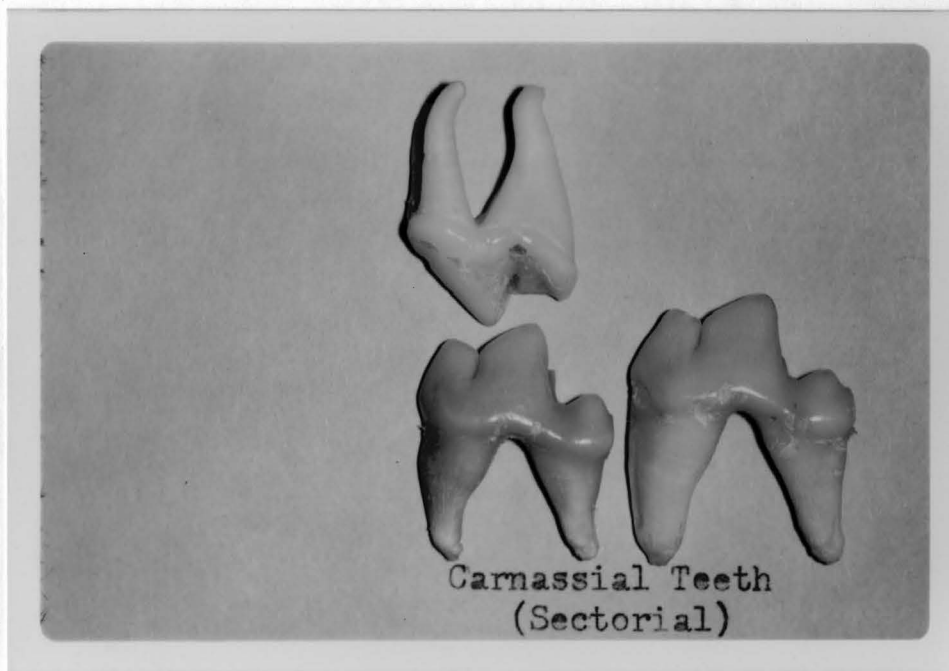
FIGURE 16

I.D. #322: OCCLUSAL WEAR PATTERN

- Note:
1. Pattern evident on mesial inclines of both Max. 4th premolars. An exposed horizontal bar on this animal's cage bore obvious tooth marks.
  2. Although the above max. 2nd and 3rd premolars were intended to be a part of this study, the animal's habitual gnawing produced their obvious fractures. All were excluded from this investigation.

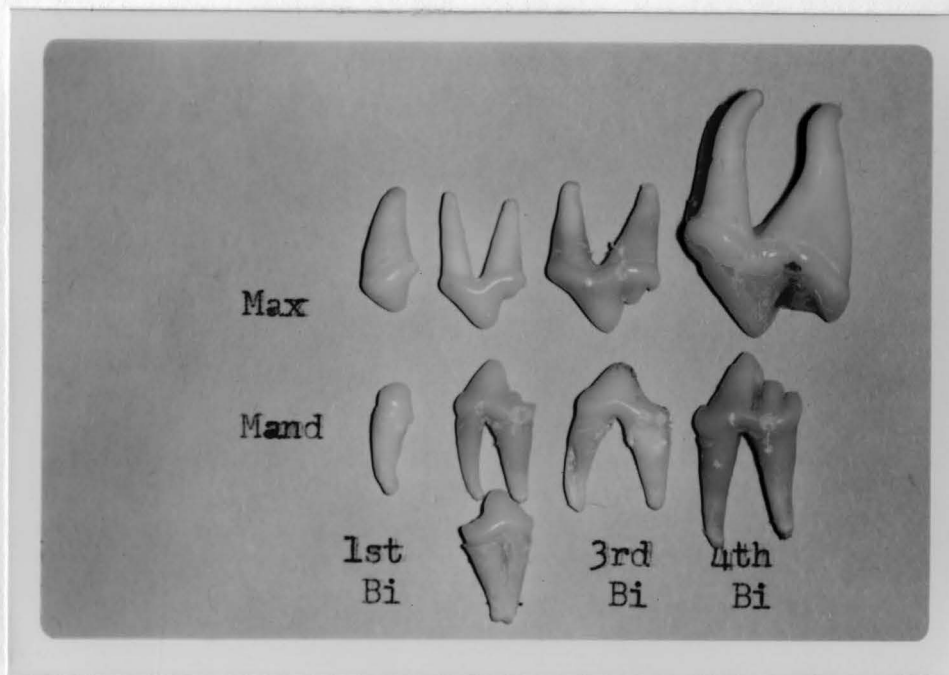


FIGURE 17

CARNASSIAL TEETH OF DOGS

- Note:
1. The upper specimen is a Max. 4th premolar.
  2. The lower tooth is a Mand. 1st molar.
  3. Developmental grooves on interradicular surface of root.

FIGURE 18

DOG MAXILLARY AND MANDIBULAR PREMOLARS

- Note:
1. An increase in root length with each succeeding posterior tooth.
  2. The mand. 2nd premolar roots may be less divergent than the roots of the mand. 3rd premolar. An example of fused mandibular 2nd premolar roots is included.
  3. The distal root is usually shorter (1-2mm.) and has more bulk than the mesial root on the same tooth.





## APPROVAL SHEET

The thesis submitted by Dr. Donald L. Scoralie has been read and approved by members of the Department of Oral Biology.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is, therefore, accepted in partial fulfillment of the requirement for the degree of Master of Science.

May 22, 1972  
Date

John V. Madonia D.D.S. Ph.D.  
Signature of Advisor